

**DENTAL CALCULUS:  
COMBINING CURRENT METHODS IN THE STUDY OF DIET AND  
MOUTH USE ACTIVITIES AMONG NEOLITHIC AND EARLY BRONZE  
AGE HUNTER-GATHERERS OF THE CIS-BAIKAL, SIBERIA**

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By

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## **Abstract**

The utility of dental calculus as a proxy for diet and mouth use is explored for the Middle Holocene Cis-Baikal region of Central Siberia based on two methods: a macroscopic analysis of severity and a microscopic analysis of particles within deposits. The study area was inhabited by two culturally and biologically distinct cultures, the Early Neolithic (EN) Kitoi culture (8,000 to 7,000/6,800 cal B.P.) and the Late Neolithic-Early Bronze Age (LN-EBA) Isakovo-Serovo-Glaskovo (ISG) cultural complex (6,000/5,800 to 4,000 cal B.P.), separated by a period of cultural transition marked by a cessation in formal cemetery use. Data were collected from four cemetery sites, two dating to the EN and two dating to the LN-EBA. Nonparametric testing of calculus severity revealed that, for adult males and juveniles, lakeshore populations displayed greater affinity to each other than to their contemporaneous cultural counterpart populations living along riverine systems in the Angara River Valley. Trends within the EN cemetery Shamanka II contrasted to the other cemetery populations, with noticeably larger deposits in anterior quadrants and significant sexual distinctions. The proportion of protein to carbohydrates consumed is known to influence calculus formation, but both cultural groups lived on a diet based predominately on meat sources so dietary ratios alone do not adequately explain the differences distinguished. A complex multifactorial model involving microregional differences in resources/environment, foraging patterns, individual variation, and dental wear patterns provides at least a partial explanation for the results observed. A wide range of particles were recovered during the microscopic analysis of calculus, albeit in low concentrations. The low starch grain counts were consistent with a diet based predominately on meats but still provide some of the first direct evidence for plant consumption in the Cis-Baikal, including possible plant processing by cooking or grinding based on damage evident on the grains. Other particles recovered may provide evidence of mouth use activities or palaeoenvironmental influences. Together, the two components of this analysis offer strong evidence that dental calculus is a useful tool for reconstructing hunter-gatherer lifeways but also highlight the limitations of conducting this type of research on previously excavated and potentially contaminated material.

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# **Chapter 1**

## **Introduction and Background to the Cis-Baikal**

### **1.1 Introduction**

This research was conducted as part of the Baikal-Hokkaido Archaeology Project (BHAP), a large multidisciplinary project housed at the University of Alberta and funded by the Social Sciences and Humanities Research Council of Canada (SSHRC). The project's goal is to investigate middle Holocene hunter-gatherer cultural dynamics in two different regions of Northeast Asia: Siberia's Cis-Baikal (Russian Federation) and Japan's Hokkaido Island (Weber et al. 2012). In general, hunter-gatherer sites are comprised of a large number of short term campsites with small artifact assemblages that are difficult to interpret due to the high mobility of these populations (Weber et al. 2010b). In contrast, these study areas feature abundant archaeological materials from both habitation sites and cemeteries with large collections of well-preserved human remains that date to various periods during the Holocene (Weber and Bettinger 2003; Weber et al. 2012). As such, they provide a unique opportunity to study northern prehistoric hunter-gatherers. The BHAP strives to create a better understanding of the specific mechanisms driving the local trajectories of these regions by analyzing their similarities and differences; studies of the human remains do so through a multidisciplinary "individual life history" approach (Weber and Bettinger 2003; Weber et al. 2012). This approach creates opportunities for archaeologists to reconstruct long segments of life histories from birth to death, to assess variation in prehistoric human behavior, and to place this behavior in the context of dynamic interactions within socio-cultural and environmental settings (Weber et al. 2012; Zvelebil and Weber 2013). This approach has been utilized within the BHAP through collaborative research including bioarchaeological analyses (i.e., the comprehensive examination of human osteological remains), as well as stable isotope, genetic, palaeoenvironmental, and faunal and artifact analyses (e.g., Faccia et al. 2014; Gibbs and Jordan 2013; Katzenberg and Weber 1999; Katzenberg et al. 2009, 2012; Lieverse et al. 2007a, 2007b, 2008, 2013; Losey et al. 2008, 2014; MacKay et al. 2012, 2013; McKenzie 2006; Mooder et al. 2005, 2006; Nomokonova et al. 2013; Scharlotta et al. 2013; Tarasov et al. 2007; Temple et al. 2014; Weber et al. 1998, 2006, 2010a; Weber and Goriunova 2013; Waters-Rist et al. 2011; White 2006;



White et al. 2013; White and Bush 2010). This study focuses only on materials from the Cis-Baikal region.

BHAP's Cis-Baikal study area was inhabited from 8,000 to 7,000/6,800 calibrated radiocarbon years before present (cal B.P.) by the Early Neolithic (EN) Kitoi culture, after which there was a dramatic cultural transition, marked by a deficiency in archaeological materials and a cessation of formal cemetery use, for a period of 800 to 1,000 years (Weber et al. 2002). This "hiatus" simply refers to this period's lack of formal cemeteries and does not mean that the area was uninhabited during this time. Following this hiatus period, a culturally and biologically distinct population known as the Isakovo-Serovo-Glaskovo (ISG) cultural complex inhabited the area during the Late Neolithic and Early Bronze Age (LN-EBA) period, extending from 6,000/5,800 to 4,000 cal B.P. (Weber 1995; Weber et al. 2002; Weber and Bettinger 2010). One of the primary goals of the BHAP is to study the development of hunter-gatherer adaptations on either side of the hiatus (Weber et al. 2010b). Research goals include answering questions related to: (1) the spatial and chronological patterns of subsistence, including diet and mobility; (2) patterns of social relations, including age structure, gender and age roles, and intercommunity interactions; (3) the environmental context of biocultural changes; (4) inter- and intracultural biological relatedness; and (5) mortuary ritual and world views (Weber et al. 2010b:xvii).

This thesis will focus on contributing new information primarily to the first research goal/question, by seeking to reconstruct aspects of diet and mouth use activity in the Cis-Baikal through the analysis of dental calculus, mineralized plaque that adheres to tooth surfaces (Hillson 1996). Four cemetery sites will be examined: two dating to the EN, Shamanka II and Lokomotiv, and two dating to the LN-EBA, Ust'-Ida I and Khuzhir-Nuge XIV. These are some of the largest and most well documented cemetery sites in the Cis-Baikal. Dental calculus can provide a wealth of information about the dynamics of past cultures including the types of food consumed, oral health, and individual variation such as age at death and sex (Lieverse 1999). Since dental calculus formation is multi-causal (Lieverse 1999), it also has the potential to add information on environment and biological relatedness, among other things.

The remainder of this chapter outlines the geographical, ecological and archaeological context of the Cis-Baikal region and provides background on the cemetery sites used in this analysis. Chapter 2 provides background information on the composition and aetiology of plaque and dental calculus and how the latter can be useful within archaeological contexts. The specific

research questions for this thesis are outlined at the end of chapter 2. Next, Chapter 3 offers a detailed report of the methodology used in this analysis. Chapters 4 and 5 present the results, discussion and interpretation of the macroscopic analysis of dental calculus severity within and among the cemetery populations and the microscopic analysis of microparticles within the calculus deposits of select Cis-Baikal individuals, respectively. Lastly, the conclusions of this research project, as well as directions for future research on these subjects, are detailed in Chapter 6.

## 1.2 Geographical and Ecological Context

The following summary is based foremost on the work of Kozhov (1950, 1963, and 1972), as summarized by Weber (2003). Weber's (2003) summary will be cited for the remainder of the section. Additional sources are cited where appropriate. The Cis-Baikal region of Siberia, Russian Federation, is located to the north and west of Lake Baikal and spans from 52° to 58°N latitude and 101° to 110°E longitude (Figures 1.1 and 1.2; Weber 2003; Weber and Bettinger 2010). The region includes the basin of the Angara River from its source at Lake Baikal north to Ust'-Ilimsk, the drainage of the upper Lena up to Kirensk, and the west coast of Lake Baikal including Ol'Khon Island (Figure 1.2; Michael 1958; Weber et al. 2002).

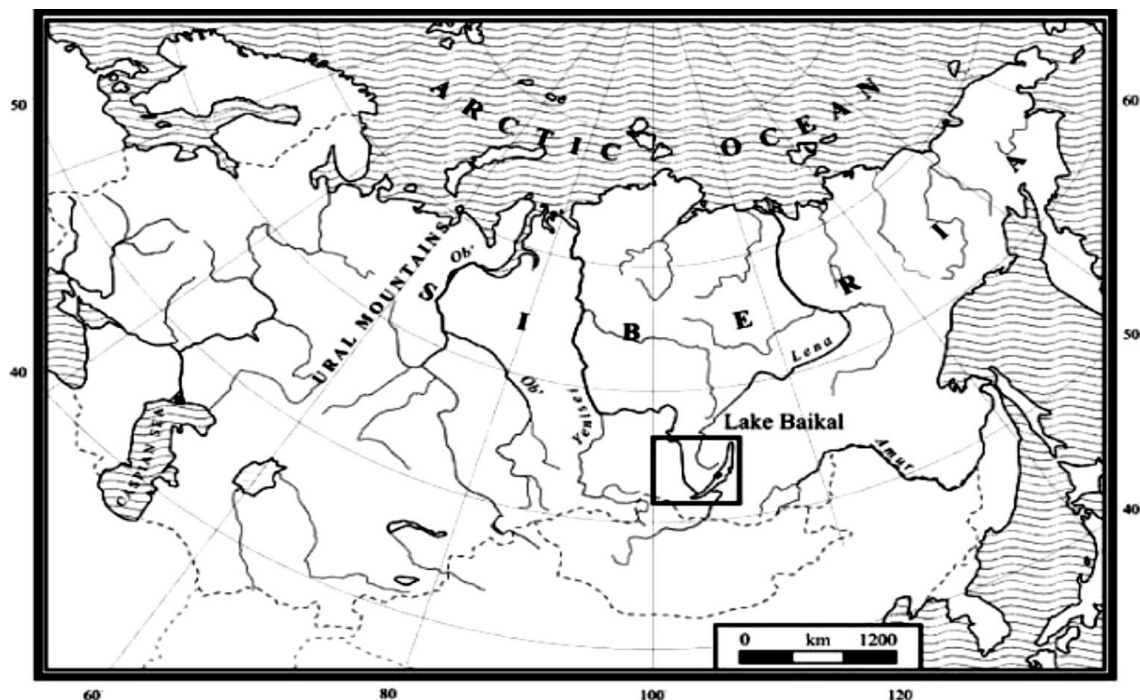


Figure 1.1 Map of Siberia, highlighting the Cis-Baikal region (courtesy of the BHAP).

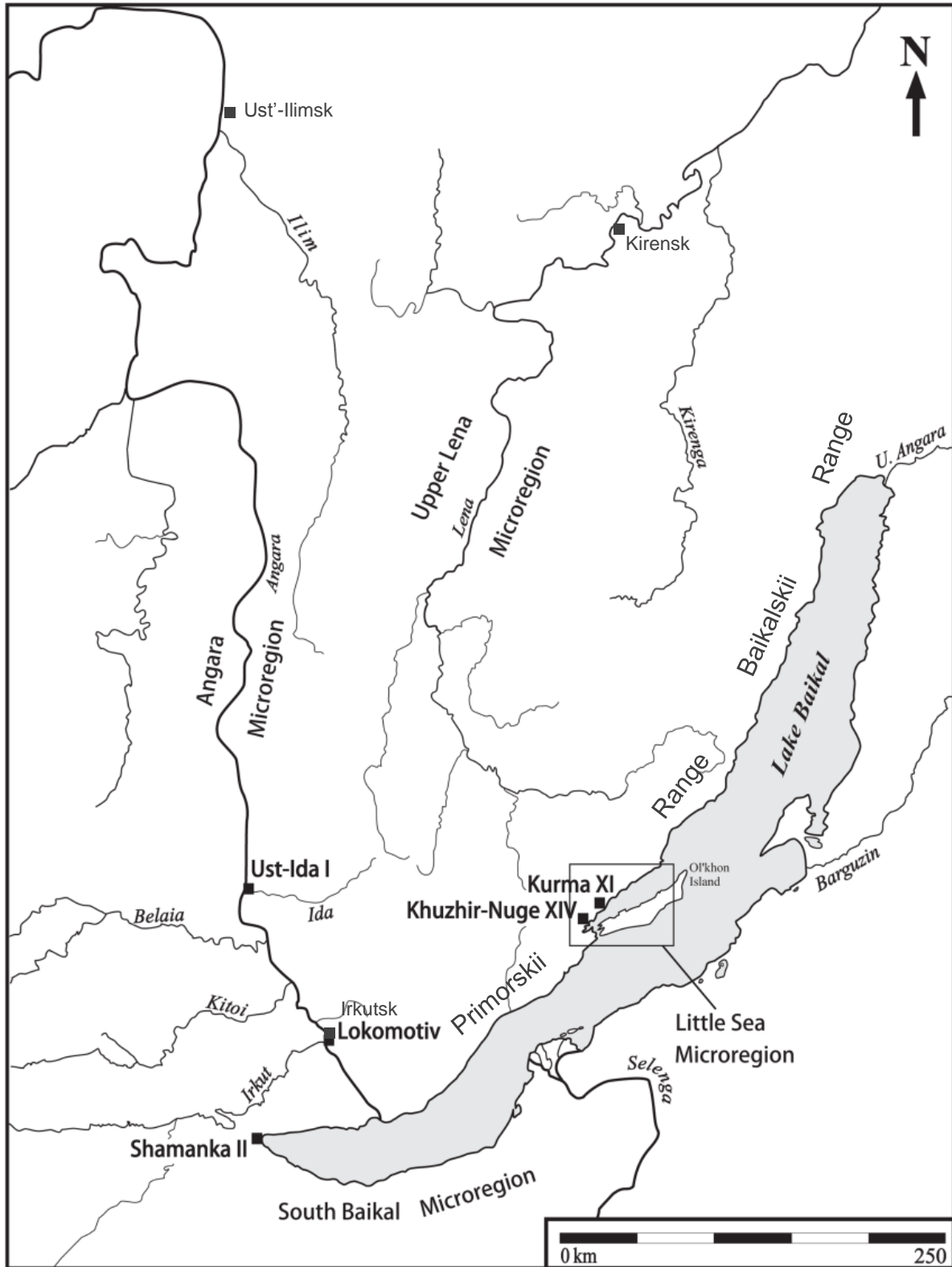


Figure 1.2 Microregions and cemeteries in the Cis-Baikal (modified, courtesy of the BHAP).

Lake Baikal is the deepest freshwater lake in the world, reaching depths of approximately 1635 m, and contains 20 to 25 percent of the world's freshwater reserves (Kozhov 1963; Weber et al. 2011). Three mountain ranges exist within the Cis-Baikal region. The Sayany Mountains, to the southwest of Lake Baikal, are the highest in the region, reaching heights of 3,200 m above sea level, while the Primorskii and Baikalskii ranges line the lake's west and north shores (Figure 1.2; Weber 2003). The area south and east of Lake Baikal is known as the Trans-Baikal region. Together the Trans- and Cis-Baikal regions form the Baikal Mountain Region (Weber 2003).

The Cis-Baikal region has a markedly continental boreal climate, with long, cold, and dry winters and short, relatively hot, and dry summers (Weber 2003; Weber et al. 2002). Variability in temperature, topography, geology, hydrography, precipitation, vegetation, and terrestrial and aquatic fauna result in a mosaic of environmental conditions and divide the region into distinct microregions (Weber 2003; Weber et al. 2002; Weber et al. 2012; Weber and Bettinger 2010). Four main microregions are recognized by the BHAP: (1) the Angara River Valley from its headwaters at Lake Baikal to the confluence with the Ilim River; (2) the upper Lena River Valley to the mouth of the Kirenga River; (3) the Little Sea, along the main section of Baikal's northwestern shores, including Ol'khon Island; and (4) South Baikal, which extends from the delta of the Selenga River to the western coast of Lake Baikal (Figure 1.2; Weber and Bettinger 2010:492).

The Angara River Valley microregion is dominated by thick taiga forests, consisting mostly of pine (*Pinus sp.*) and larch (*Larix sp.*) (Galazii 1993; Weber 2003; McKenzie 2006), in its downstream sections; these begin ~380 kilometers from Lake Baikal (Weber 2003). It also features pockets of steppe-forest, connected by stretches of open vegetation in its upstream sections (Weber and Bettinger 2010; Weber et al. 2011; Weber et al. 2012). From the standpoint of human subsistence, important herbivores that typically inhabit this ecotone include: red deer (*Cervus elaphus*), which prefer forest-steppe and taiga landscapes; roe deer (*Capreolus capreolus pygargus*), in the open landscapes such as the steppe and forest-steppe; moose (*Alces alces*), which prefer the taiga; and musk deer (*Moschus moschiferus*), in the highlands and montane taiga (Weber 2003). Important fur-bearers include sable (*Martes zibellina*), squirrel (*Sciurus vulgaris*), hare (*Lepus timidus*), Siberian polecat (*Martes sibiricus*), fox (*Vulpes vulpes*), ermine (*Martes ermine*) and chipmunk (*Eutamias sibiricus*). Other mammals typical in the Cis-

Baikal boreal forest include boar (*Sus scropha*), otter (*Lutra lutra*), brown bear (*Ursus arctos*), wolf (*Lupus lupus*), lynx (*Felis lynx*), and bobcat (*Felis rufus*) (Weber 2003).

Hydrologically, the Angara drainage basin, part of the larger Yenisei River drainage system, is divided into four main sections: the upper section (71 km in length), two middle sections (240 and 680 km), and the lower section (860 km; this section is outside of the study area and will not be discussed further) (Weber 2003). The Angara begins at Lake Baikal and eventually flows into the Arctic Ocean, via the Yenisei River. The river's important fisheries are independent from each of the other fisheries to be discussed below, except at the mouth of the Angara, where there is some overlap with Lake Baikal basin fisheries (Weber 2003). The Angara fishery is highly productive, but the dynamics of the system change within the different sections of the drainage basin. In the upper section, the ecology of the fishery is highly influenced by Lake Baikal, as the river is the lake's only outlet. Baikal black grayling (*Thymallus arcticus baicalensis*) are the most abundant fish species in this microregion, with fish such as lenok (*Brachymystax lenok*) and taimen (*Hucho taimen*) also present but less abundant. In the middle sections, tributary rivers begin to contribute more and more water, and the ecology of the system changes to something that more closely resembles other large Siberian rivers. At the beginning of the second section there is more variation in fish species, including Siberian dace (*Leuciscus leuciscus*), arctic grayling (*Thymallus arcticus*), freshwater perch (*Perca sp.*), northern pike (*Esox lucius*), burbot (*Lota lota*), roach (*Rutilus rutilus lacustris*), humpback whitefish (*Coregonus pidschan*), lenok, and taimen. In the second portion of the middle section, the ecology changes again, with humpback whitefish, Siberian sturgeon (*Acipenser baerii*), Siberian starlet (*Acipenser ruthenus ruthenus*), Siberian dace, and arctic grayling being more abundant. None of these fish species reach Lake Baikal in their migration routes (Weber 2003; McKenzie 2006).

The upper Lena River Valley microregion features vegetation very similar to that found in the Angara Valley microregion, but dominated by larch (Galazii 1993). The Lena is the longest Siberian river – approximately 4,500 km. However, only the upper 650 km fall within the Cis-Baikal study area (Weber 2003). It begins from small marshy creeks on the western slopes of the Baikalskii Mountain range, rather than draining from Lake Baikal; it is therefore the only drainage within this study that is completely separate from Lake Baikal. It is characterized as a mountain river in its upper sections, maintaining many of its montane characteristics for the next

500 km of its course, but also accumulating a much larger volume of water and developing a wider trough (Weber 2003). The downstream sections feature taiga forests dominated by larches, as well as patches of steppe-forest. There are also stretches of open vegetation in the upstream regions (Weber and Bettinger 2010). Terrestrial resources are quite similar to those listed above for the Angara Valley, with reindeer (*Rangifer tarandus*) and Siberian mountain goats (*Capra sibirica*) also present in the highlands and alpine areas of the Baikalskii Mountains (Weber 2003; Weber and Bettinger 2010).

Less information is available for the upper Lena fisheries, but the area is known to feature a typical but impoverished Siberian river fish complex. Fish species include taimen, lenok, ide (*Leuciscus idus*), Lena tugun (*Coregonus tugun*), arctic grayling, Siberian dace, roach, northern pike, perch, and burbot. Fish productivity is substantially lower than the Angara fisheries (Weber 2003; McKenzie 2006).

The Little Sea microregion, also known as the Ol'khon area, consists of Baikal's largest island, Ol'khon, as well as a 120-km section of the lake's northwest coast across from the island, the shallow water body between the island and the coast, referred to as the Little Sea, and the smaller islands within it (Weber 2003). The Little Sea microregion receives much less precipitation than the other microregions annually and an open steppe dominates the coast in this area and much of Ol'khon Island (Weber et al. 2012). High winds and scarce vegetation also result in very little snow accumulation in the winter. Roe and red deer are generally plentiful within this region (Weber et al. 2012; Weber 2003).

The Little Sea exists within the Lake Baikal fishery basin and contains some of the richest littoral and pelagic fish resources in the whole lake (Kozhov 1963). This richness reflects its diverse nature, with numerous shallow coves to the south and deep pelagic zones to the north (Weber 2003). Omul (*Coregonus autumnalis migratorius*), Baikal whitefish (*Coregonus lavaretus baicalensis*), perch, and northern pike are all abundant within this region (Kozhov 1963; Sorokin and Sorokina 1988; Weber 2003; McKenzie 2006). Baikal seal (*Pusa sibirica*) is another important resource that would have been available seasonally to hunter-gatherers only on Lake Baikal (Weber and Bettinger 2010), though not within the Little Sea itself.

In the past, BHAP research focused on the three regions just described, but the South Baikal microregion has more recently been added (Weber and Bettinger 2010). The area is dominated by open landscape of the type preferred by roe and red deer, and it encompasses the

southern shores of Lake Baikal from its western tip to the Selenga River delta to the east and the Tunka region to the west of Lake Baikal (Weber and Bettinger 2010).

The South Baikal microregion is part of the Lake Baikal basin fishery. The south coast of Lake Baikal consists of a large littoral zone with abundant whitefish and black and white grayling, as well as omul in the pelagic zones (Kozhov 1950; Weber and Bettinger 2010). As with other parts of Lake Baikal, seal would have been available seasonally in this region (Weber and Bettinger 2010).

Of final note, a few plant types available throughout the Cis-Baikal region are known to be of economic value. Siberian pine (*Pinus sibirica*) yield large quantities of tiny shelled nuts, known as pine or cedar nuts, which provide both a food and oil source for local populations (McKenzie 2006; Weber et al. 2002). Edible mushrooms and berries are also available seasonally (Weber et al. 2002). While a wide range of other plant species are present in the study area, these are the only three plant resources possibly contributing to the diet of middle Holocene hunter-gatherers noted by the BHAP to date (Weber et al. 2002). Distributions within specific microregions have not been mentioned in this literature so it is not known if these resources occur within some microregions more than others.

### **1.3 Archaeological Context**

The Neolithic in the Cis-Baikal was marked by the advent of ceramic technology, the bow and arrow, and ground stone tools. The early Bronze Age was characterized by the appearance of copper and bronze artifacts (Weber 1995). It should be noted that this is distinct from areas of western Eurasia, where the Neolithic was associated with the introduction of agriculture and sedentism (Weber et al. 2002; Chazan 2008:208).

Archaeological research in the Cis-Baikal area began in the nineteenth century with notable excavations conducted by Vitkovskii at the site of Kitoi and by Petri at the site of Ulan-Khada (Weber 1995). In the 1950s A. P. Okladnikov published a new culture history for the region, based on excavation data from the nineteenth century up until the beginning of World War II (Okladnikov 1950, 1955). It drew extensively from his own work in the region and focused on a unilinear progression of complexity in technology, economy, social and political organization, ritual, and beliefs (Weber 1995). Based on this, he delineated four successive cultural groups: the Isakovo, Serovo, and Kitoi cultures in the Neolithic, and the Glazkovo

culture in the Early Bronze Age. For additional information, Weber (1995) provides a comprehensive synthesis of Okladnikov's original work in English.

Okladnikov's model was heatedly debated in the following years, mostly with regards to the placement of the Kitoi culture within the sequence (Weber 1995). Gerasimov (1955) released a critique of Okladnikov's model soon after it was published and many other models were proposed, most based on variations in pottery and burial material. Still, the original model held until the 1980s (Weber 1995). In 1982, Konopatskii published the first sequence of radiocarbon dates on Cis-Baikal materials, but the results were questionable due to problems with sample sizes and doubts regarding direct association of charcoal samples with burials from which they were recovered (Weber 1995; Link 1999). More reliable radiocarbon dates produced by Momonova and Sulerzhitskii (1986, 1989) effectively disproved Okladnikov's model. They found that the Kitoi culture predated the Isakovo with no overlap, while the subsequent Isakovo, Serovo and Glazkovo were sequential but overlapped in their date ranges. Calibrated versions of these dates are provided in Weber (1995), and newer dates continue to support this cultural sequence. The calibrated radiocarbon dates presented here are reported in Weber et al. 2006 and 2010a. These new dates, along with archaeological and osteological data from cemetery and habitation sites, form the basis for current understandings of the region's culture history. Recently, a significant freshwater reservoir effect was found to influence aquatic fauna at Lake Baikal, due to the intake of carbon from the lake rather than the atmosphere which caused the radiocarbon dates to overestimate the actual age of the samples (Nomokonova et al. 2013). New dates taking into account this fresh water reservoir effect are forthcoming (Weber et al. 2015).

### *1.3.1 Early Neolithic (EN)*

The Kitoi culture inhabited the Cis-Baikal during the EN from 8,000 to 7,000/6,800 cal B.P. Their sites display substantial regional variability and the first evidence of formal cemetery use in the region (Weber and Bettinger 2010). "Classic" Kitoi cemeteries are confined to the Angara River Valley, with the only exception being at the site of Shamanka II in South Baikal. Diagnostic mortuary characteristics of the "classic" Kitoi include: the presence of red ochre, head-to-toe orientation in double and communal graves, bodies in an extended supine position and grave goods such as composite fishhook shanks, canines of the Siberian marmot (*Marmota sibirica*), calcite rings, and arrowheads with an asymmetrical concave bases (Bazaliiskii 2003; Weber and Bettinger 2010). Cemeteries that do not display these characteristics have also been



found dating to the EN, especially in the Little Sea. Despite their difference, these sites are still considered part of the same EN complex. This pattern suggests restricted cultural exchange, or socially closed communities, among groups during this period (Weber et al. 2002; McKenzie 2003; Weber and Bettinger 2010).

EN cemetery samples display intersite/microregional variability in stable isotope signatures across the Cis-Baikal, likely reflecting heavy reliance on local aquatic resources and restricted home ranges (Katzenberg and Weber 1999; Weber et al. 2011; Haverkort et al. 2008; Weber and Bettinger 2010), with the only exception being between the cemeteries Lokomotiv, in the Angara River Valley, and Shamanka II, along the south Lake Baikal shore, where greater similarity in isotope ratios has been noted (Katzenberg et al. 2010; Weber and Bettinger 2010). Katzenberg and Weber (1999) suggest that this means that groups in the Angara and Lena were not regularly travelling to Lake Baikal for resources and instead local resources were sufficient. Kitoi populations had a tendency to establish home bases along particular waterways, tethering them to certain areas within the Cis-Baikal (Weber et al. 2002). Kitoi populations had low residential mobility during the fishing season, which would have led to shortages in other resources not available at the home base. Small hunting parties would have been designated to procure these other resources from areas beyond the home base, a strategy known as logistical foraging. Residential mobility likely increased during the fishing off-season (Weber et al. 2002).

Archaeological artifacts found within burials and the location of major cemeteries near water sources provide further evidence of a specialization in freshwater resources during the EN (Weber and Bettinger 2010). Lieveise et al. (2009) suggest that enthesal changes to the upper limbs of EN males are consistent with muscular strains caused by rowing and may indicate that water craft were utilized, though this is not supported with archaeological evidence of this technology. Seal would have been available seasonally to populations along the Lake Baikal shores (Weber et al. 1993; Katzenburg et al. 2010).

Ungulates were another important resource for the Kitoi. Initial stable isotope data suggested that terrestrial resources played a smaller role in the EN compared to later periods but further analysis has revealed that the consumption of certain fish was masking the effects of ungulate signatures in the samples (Katzenberg et al. 2010). Therefore there is no reason to conclude that terrestrial game played a less important role in the EN compared to the LN-EBA (Weber and Bettinger 2010), except possibly in the Angara River Valley microregion, where

stable isotope data has clearly indicated greater consumption of fish than populations in later periods (Weber et al. 2011). Little information is available on plants in the diet at this time (Weber et al. 2002).

All classic Kitoi cemeteries in the Cis-Baikal are categorized as community cemeteries (Weber and Bettinger 2010). McKenzie (2010) defines community cemeteries as cemeteries where a substantial portion of the population is eligible to be interred therefore these cemeteries exhibit non-selective demographic profiles. The BHAP has analyzed data on cemetery demographics, graves and individuals in an attempt to determine overall population size (Weber and Bettinger 2010), in the absence of substantive information from habitation sites. While there are problems with this approach, Weber and Bettinger (2010) suggest that concurrent community cemeteries provide an acceptable proxy for the number of community groups within the Cis-Baikal region. Kitoi cemeteries are generally larger but less numerous than in later periods and these cemeteries are largest on the upper Angara and decrease in size considerably downstream. Therefore, the Kitoi likely had a large but unevenly distributed population (Weber and Bettinger 2010).

Osteological research has provided important information on stress, health, and activity patterns during the EN. Studies on osteoarthritis reveal that adult males exhibited significantly higher frequencies of osteoarthritis in the knee joint and spine, supporting interpretations that Kitoi males engaged in higher levels of pedestrian travel associated with logistical foraging (Lieverse et al. 2007b; Lieverse 2010). Again, this was likely in response to reduced residential mobility related to increased settlement around spatially delimited fishing resources. Paralleling this, analyses of enthesal changes to skeletal elements show that males and females exhibited significant differences for both the upper and lower limb. The patterning suggests that males and females were not engaging in dramatically different tasks, but instead males were engaged in certain tasks over longer durations and with greater intensity (Lieverse et al. 2009, 2013). In the lower limb, patterns suggest an increase in sexual disparity in tasks with increasing age, except possibly at Lokomotiv (Lieverse et al. 2013). It is thought that these sexual disparities were also related to male-dominated logistical foraging that generated higher mobility levels using lower limb muscles more intensively (Lieverse et al. 2013).

Studies focusing on enamel hypoplasia frequencies indicate slightly higher levels of physiological stress among Kitoi populations as compared to LN-EBA populations. This pattern

is potentially due to Kitoi populations' increased reliance on fish exploitation and likely periods of seasonal or annual shortages (Lieverse et al. 2007a; Lieverse 2010; Waters-Rist 2011). Periods of greater physiological stress are further supported by weaning patterns determined through stable isotope analyses (Waters-Rist et al. 2011). Waters-Rist et al. (2011) found that EN subadults were weaned at about 3.5 years of age on average whereas LN-EBA subadults were completely weaned by three years of age. Also, weaning occurred over a shorter period of time in EN subadults (one to 1.5 years in duration compared to two years in LN-EBA subadults). Many EN infants appear to have died while still breastfeeding, when breast milk should have been providing a buffer against illness and malnutrition. This pattern suggests periods of considerably increased stress impaired the effectiveness of breast milk to act as a buffer (Waters-Rist et al. 2011). Despite this, both trauma and disease frequencies were low (Lieverse 2010).

The classic Kitoi seem to have displayed a high degree of social differentiation for a hunter-gatherer group (Weber and Bettinger 2010). These differences appear to have been primarily structured based on age and sex, with adult males likely holding the dominant positions within the group (Link 1999; Weber et al. 2002; McKenzie 2003). At cemetery sites such as Lokomotiv, goods were often not equitably distributed, with some graves containing no artifacts and the richest graves containing hundreds of artifacts (Bazaliiskii 2003; Weber and Bettinger 2010). This great disparity is not seen in Little Sea EN sites though. Differences in entheses, discussed above, show disparity in the tasks conducted by males versus females (Lieverse et al. 2009, 2013). Losey et al. (2012) hypothesize that specialization in the exploitation of fish resources likely required more complex socioeconomic organization in order to exploit the resource efficiently (e.g., division of labor, coordination of surpluses and storage).

### *1.3.2 Middle Neolithic*

At about 7,000/6,800 cal B.P. to 6,000/5,800 cal B.P. there was a shift, and formal cemeteries disappear from the archaeological record. Not a single burial has been found in the region that can be firmly dated to this period (Weber 1995; Weber and Bettinger 2010; Weber et al. 2012). This period of cemetery cessation is termed the "biocultural hiatus", but it should be noted that this term only applies to formal internments and does not mean that the area was uninhabited during this time. Populations in the region are thought to have consisted of small dispersed and mobile groups engaging in some fishing and sealing and with little social differentiation (Weber and Bettinger 2010).

White and Bush (2010) suggest that this hiatus period may coincide with a period of significant climatic and environmental change in the Cis-Baikal. Palaeoenvironmental data show a general trend from warming-wet (increasing temperatures and humidity/precipitation) to warming-dry (increasing temperature but greater aridity) conditions during the Early-Middle Neolithic transition; these changes likely had an effect on both terrestrial and aquatic ecosystems. Proxy records from Lake Hovsgol, located south of Lake Baikal in northern Mongolia, indicate increasing aridity between 7,500 to 6,500 cal B.P., leading to reduced surface runoff and lower nutrient input into the lake (Prokopenko et al. 2007; White and Bush 2010). Increasing aridity may have reduced the forest cover and expanded the forest-steppe ecotone as well. To the east of Lake Baikal, pollen reconstruction from Lake Kotokel suggests a slight decrease in woody cover around the lake beginning between 7,000 and 6,500 cal B.P. and the spread of more drought resistant Scots pine (*Pinus sylvestris*) throughout the Lake Baikal region (Bezrukova et al. 2008, 2010). While still only speculative at this point, increasing aridity and temperature change could have caused a critical fluctuation in the riverine and lake ecosystems, disrupting the seasonal subsistence base of Kitoi groups. Resources could have become too erratic, less abundant and/or temporally dispersed to support large, moderately sedentary populations (White and Bush 2010). Adaptive strategies during these changing conditions would have been to increase group mobility and abandon established settlements and formal cemeteries (White and Bush 2010). Data collected from three peat bog sites around Lake Baikal, representing different climatic regions, suggest that environments/vegetation to the west of Lake Baikal (e.g., Little Sea) were more sensitive to changes in aridity than the more humid areas to the east and south (Bezrukova et al. 2013).

White and Bush (2010) also suggest that the changing climate could also possibly have had the reverse effect and, instead, these changes may have increased the range of forest vegetation, leading to richer terrestrial resources. MacKay et al. (2012, 2013) found that a compositional switch from birch to Scots pine began to occur around 9,000 cal B.P. at ESM-1, a lake located roughly 200 km east of the southern tip of Lake Baikal; a similar shift also became evident in Lake Khall (located in the Ol'-Khon region) sediments between 5,150 and 2,480 cal B.P., but they consider this event at Lake Khall to be only a forest turnover, not an afforestation event or a decrease in steppe communities.

### *1.3.3 Late Neolithic and Early Bronze Age (LN-EBA)*

During the LN and EBA (6,000/5,800 to 4,000 cal B.P.), a new and genetically distinct population inhabited the Cis-Baikal (Mooder 2005, 2006). This population is known as the Isakovo-Serovo-Glazkovo (ISG) cultural complex, and with them came the reappearance of formal cemetery use. The Isakovo, Serovo and Glazkovo cultures were previously separated within Okladnikov's model but research has established significant continuity between these three constructs; therefore the BHAP treats them as a single cultural complex (Weber et al. 2002). Despite this, there are significant differences between the LN Isakovo/Serovo cultures and the EBA Glazkovo culture. As delineated by Shepard (2012), these include aspects of child burials, grave good distribution and contents, the frequency of multiple burials, and an overall increase in burial activities. This research treats the ISG as a single entity to maintain consistency with most other BHAP research.

As outlined above, while LN and EBA cemeteries display significant variability, they also share a suite of similarities, suggesting cultural continuity. The mortuary use of fire within burial contexts is seen in both the LN and the EBA, and there appears to be a coherent spatial organization to LN and EBA burials at cemetery sites. This means that many cemeteries contain both LN and EBA burials, but the younger EBA burials rarely disturb the older LN burials, pointing to knowledge of and respect for older traditions (Weber and Bettinger 2010). LN-EBA burials are also consistently oriented with reference to adjacent bodies of water, though the exact orientation is different. For example, in the Angara River Valley, Isakovo graves are oriented parallel to the river with the head pointing upstream, Serovo graves are oriented perpendicular to the river with the head pointing away from it, and Glazkovo graves are oriented parallel to the river with the head facing downstream (Bazaliiskii 2003; Weber and Bettinger 2010). Other similarities include the use of paving stones as cairns on top of burials and inclusion of tool kits associated mostly with hunting.

Unlike the EN Kitoi, LN-EBA burials display much more regional continuity in cemetery characteristics (Weber and Bettinger 2010). Key characteristics of the LN tradition include: clay pots with net impressions, fairly low quantities of utilitarian grave goods, and a reduced number of fishing-related objects within the burial context, when compared to Kitoi burials (Bazaliiskii 2003). The main identifying features for the EBA are the appearance of copper and bronze, mostly as decorative items (e.g., rings, pendants), as well as polished ornamental white nephrite

rings and disks, a raw material which suggests long distance trade (Weber et al. 2002; Shepard 2012). Green nephrite goods (e.g., ground weapons) are often found within graves of the earlier EN and LN traditions (Bazaliiskii and Savelyev 2003; Bazaliiskii 2003), but these white nephrite rings and disks are the first evidence of nephrite use as personal adornment items in the region and they may be linked to the economic status of individuals they were interred with (Shepard 2012). Social differentiation among ISG populations is thought to have been much more equitable than in the EN, along both age and sex lines (Link 1999; Weber et al. 2002; McKenzie 2003).

Stable isotope data show that LN and EBA diets were more homogeneous than in the EN; spatially separated groups consumed fish and mammal species with more similar stable carbon and nitrogen ratios than EN cemetery populations (Katzenberg et al. 2010; Weber and Bettinger 2010). This suggests that LN-EBA groups had greater residential mobility than EN groups, utilizing larger areas within season rounds (Weber et al. 2002; McKenzie 2003). Despite this, groups are separated into two distinct clusters (Katzenberg et al. 2010). One group seems to have subsisted on a diet mainly of terrestrial game and fish; for convenience this is referred to as the game-fish or GF diet. The other group shows higher nitrogen ratios, suggesting a diet of terrestrial game combined with aquatic foods high in nitrogen, with seal figuring prominently; for this reason, this is designated the game-fish-seal or GFS diet. Inhabitants of the Little Sea display signatures for the GF and GFS diets, while inhabitants of the Angara and Lena only display GF signatures (Katzenberg et al. 2009, 2012; Weber et al. 2011; Weber and Goriunova 2013; Weber and Bettinger 2010). Within the site of Khuzhir-Nuge XIV, located in the Little Sea, individuals exhibiting these two diets are separated spatially. Individuals from the western cluster of burials are considered “locals” based on strontium isotopes and exhibit only the GFS diet, but individuals from the eastern clusters are considered likely nonlocal immigrants from the Angara and Upper Lena based on strontium analysis and exhibit both the GFS and GF diet (Katzenberg et al. 2009; Weber et al. 2011; Weber and Goriunova 2013). LN and EBA groups do not appear to have specialized in the exploitation of fish to the same extent as the earlier EN inhabitants (Weber and Katzenberg 1999), though fish and seal were still emphasized to a greater extent in the Little Sea (Katzenberg et al. 2012). Again, little information is available on plants in the diet.

The presence of two distinct dietary patterns, with no evidence of the GFS diet outside of the Little Sea, indicates that communities remained firmly established within their respective microregions, even though regional continuity in burial features suggests that groups within the microregions were likely socially connected (Weber and Bettinger 2010; Weber and Goriunova 2013). The possible exception to this is in the Little Sea region. Weber and Goriunova (2013) note that, based on strontium analysis and the presence of both GF and GFS diets in the Little Sea, people seem to have been moving to the area from other microregions such as the Angara and Upper Lena but not vice-versa. The overall LN-EBA population within the Cis-Baikal is thought to have been larger, but more widely dispersed, than in the EN (Weber and Bettinger 2010).

Osteological data provide additional information about the dynamics of LN-EBA populations. Enamel hypoplasia frequencies show that LN-EBA populations generally had lower physiological stress, suggesting more consistency in access to resources, than in the EN (Lieverse et al. 2007a; Lieverse 2010; Waters-Rist 2011). Osteoarthritis distribution and frequency studies indicate that males and females participated in activities/tasks more equitably than in the EN (Lieverse 2010), though enthesal data suggest an increase in sexual disparity in tasks related to the lower limb with increasing age (Lieverse et al. 2013). This difference was not seen in upper arm usage though (Lieverse et al. 2009). This may reflect slight differences in tasks affecting the skeleton cumulatively over time or shifts in male/female roles with advanced age. Trauma, infectious diseases, neoplasms, and dental caries were not common in these populations. This further supports that the populations experienced low physiological stress and little interpersonal violence (Lieverse 2010), excepting a small group of EBA individuals found at site Shamanka II. Lesions on these individuals suggest that they were brutally killed, possibly by an intrusive population (Lieverse et al. 2012; Shulting et al. 2015). Research on biomechanical robusticity and loading show a trend toward decreasing upper and lower limb robusticity from the EN to the LN-EBA. This attests to a change in activity patterns, possibly a decrease in intensive terrestrial mobility (Lieverse et al. 2011; Stock et al. 2010). Loading and enthesal changes suggest potential water craft use, as in the EN, though much less so in ISG females (Lieverse et al. 2011).

## 1.4 Site Description

For this research project, four cemetery populations were selected for analysis. These cemeteries are some of the largest, most diagnostic and well-documented cemeteries in the Cis-Baikal region. Two of the cemeteries, Lokomotiv and Shamanka II, are firmly dated to the EN and two, Ust'-Ida I and Khuzhir-Nuge XIV, are dated to the LN-EBA.

### 1.4.1 Lokomotiv

Lokomotiv is a large EN cemetery site located within the Angara River Valley microregion, on the upper west terrace of the Angara River at the confluence of the Irkut River in downtown Irkutsk, and about 70 km north of Lake Baikal (Bazaliiskii 2003; Figure 1.2). The site is situated about 120 m above the river level, and the graves are scattered over an area of approximately 50,000 m<sup>2</sup> (Link 1999; Bazaliiskii 2010).

The cemetery was first discovered in 1897 when evidence of ochre-painted graves was uncovered during the construction of the Trans-Siberian Railway (Ovchinniov 1904; Bazaliiskii and Savelyev 2003). Excavations were conducted by Gerasimov in 1927, Khoroshykh and Okladnikov from 1946 to 1959, and Bazaliiskii from 1980 to 1996 (Bazaliiskii and Savelyev 2003).

All excavated burials are considered to be associated with the “classic” Kitoi culture (Link 1999). Eighty-seven graves have been excavated to date, though only 82 of these were well documented. One of these burials was a tundra wolf interment, with a single male human skull, first and second vertebrae associated, dated to the beginning of the EN cemetery activity (Bazaliiskii and Savelyev 2003). Crania were missing from 29 skeletons, along with the first and second vertebrae. This pattern likely represents intentional decapitation, although it is unknown if this occurred ante- or postmortem (Bazaliiskii and Savelyev 2003; Waters-Rist 2011). Potentially another 100 graves were destroyed during past construction projects (Bazaliiskii 2003; Bazaliiskii and Savelyev 2003). It is estimated that as many as 50 graves may remain undisturbed in the park present at the site's location now (Bazaliiskii and Savelyev 2003).

All graves excavated were simple pits, and most were oval in shape, though a few rectangular burials were present as well. Individual burials were the most common (61.4%), but double (21.4%) and multiple (17.1%) interments also occurred (Bazaliiskii 2003). Together, the excavated burials contained a total of 101 individuals (Bazaliiskii 2010; Lieverse 2005).



Lokomotiv is considered to be a “community” cemetery, based on McKenzie’s definition outlined above (2010). There were a slightly larger number of males present than females and a high percentage of adults compared to juveniles, considered typical for the EN (Bazaliiskii 2003). Both human bone and grave goods were in good condition/completeness when recovered (Bazaliiskii and Savelyev 2003).

#### *1.4.2 Shamanka II*

Shamanka II is the largest excavated cemetery site in the Cis-Baikal and is located at the very southernmost tip of Lake Baikal (Figure 1.2), in the South Baikal microregion. It is situated about 22 to 27 m above the lake on the west facing slope of a narrow peninsula known as Shamanskii Mys (Bazaliiskii 2010). The site was discovered when the slope on which the site was situated began to erode, exposing bones on the cliff edge (Antonova 2011). The site was excavated by Tivanenko in 1962 and 1965, Kharinskii and Turkin in 1998 and 1999, and Bazaliiskii from 2000 to 2008 (Bazaliiskii 2010). The site is now considered to be completely excavated.

Most burials found in this cemetery date to the EN and are considered to represent the “classic” Kitoi (Weber and Bettinger 2010), though 11 produced radiocarbon dates outside of this date range; these outlying burials were not included in the current analysis. A total of 155 EN individuals were represented (Lieverse 2005). Most burials were single internments but double and multiple burials were not uncommon. Burials were typically extended and supine, with the heads pointed to the northeast (Bazaliiskii 2003, 2010). The preservation of remains was very good at this site, with low bone fragmentation rates. Shamanka II is considered to be a community cemetery, typical for EN populations (Weber and Bettinger 2010).

#### *1.4.3 Ust'-Ida I*

Ust'-Ida I is located in the Angara River Valley microregion, on a terrace about 20 m high on the east bank of the Angara River, at its confluence with the Ida River. It is approximately 250 km northwest of Lake Baikal (Bazaliiskii 2003; Figure 1.2). The first grave was excavated in 1956 by Okladnikov; then, in 1986 and 1987, high water levels from spring runoff exposed a number of LN and EBA graves. Salvage excavations conducted by Fetisov and Lemeshchenko occurred as a result of these exposures, followed by more intensive archaeological work conducted by Bazaliiskii from 1987 to 1996 (Bazaliiskii 2003).

Cultural remains from the EN through to the EBA have been found up to the present time. One grave and one ritual feature dated to the EN, 33 graves and six ritual features dated to the LN, and 17 graves dated to the EBA (Bazaliiskii 2003; Bazaliiskii 2010). A total of 67 LN-EBA individuals were represented (Lieverse 2005). This represents the largest cemetery in the Cis-Baikal associated with the Isakovo tradition (Bazaliiskii 2010). LN and EBA individuals were combined as one population for this study, as was explained above. The majority of the graves represented single internments, but double and multiple burials were found, as well. The LN graves were clustered into two discreet areas containing rows that generally ran east-west. Bodies were interred in extended and supine position and the heads pointed upstream along the Angara River (Bazaliiskii 2010). EBA burials were either in an extended and supine position, or were flexed, with heads oriented north (Tiutrin and Bazaliiskii 1996 in Waters-Rist 2011). The graves were covered with rounded paving stones (Bazaliiskii 2010).

Ust'-Ida I is often considered to be a "specialized" cemetery, where the cemetery population likely does not reflect the population demographics of the groups inhabiting the area at the time. The cemetery therefore may have had a restricted purpose or membership (McKenzie 2010, Weber and Bettinger 2010). Ust'-Ida I was dominated by the remains of juveniles, making up about 58% of the burials (Lieverse 2005; Bazaliiskii 2010:Table 3). Males occurred in slightly larger frequencies than females, typical of other sites in the Cis-Baikal (Lieverse 2005).

#### *1.4.4 Khuzhir-Nuge XIV*

Khuzhir-Nuge XIV is considered to be one of the largest completely excavated mortuary sites in the Cis-Baikal (Weber et al. 2007; Goriunova and Novikov 2010). It is located on the west coast of the Little Sea microregion near the southern end of Ol'khon Island, approximately 3 km southwest of the mouth of the Sarma River (Weber et al. 2007; Figure 1.2). It is situated on a southeastern slope overlooking a shallow bay. Overall, the sites dimensions are about 200 by 30 m (Weber et al. 2007). Excavations took place over the course of five field seasons from 1997 to 2001, directed by Goriunova and Weber (Weber et al. 2007).

Seventy-nine graves have been excavated; most of these were single internments, but seven double and two triple internments were found as well. A total of 89 individuals were recovered (Weber et al. 2007). One grave, Burial 7, was identified as a LN Serovo burial, while all other burials were consistent with the EBA Glazkovo tradition. The Serovo burial was not included in the current analysis.

Graves were divided into three well-defined clusters, with differences in grave goods, disturbance level, and extent of burning (McKenzie et al. 2008; McKenzie 2010). McKenzie (2010) interprets this as evidence of intra- rather than intergroup distinctions, and considers Khuzhir-Nuge XIV to be a “community” cemetery. There were a high proportion of individuals with undetermined sex due to poor preservation and associated high bone fragmentation; this issue obscured some demographic information (Lieverse et al. 2006). Many individuals also lacked sufficient observable features to determine age and could only be specified as “adult” (20+ years). Thirty-one percent of graves had been disturbed in antiquity (Lieverse et al. 2006).

All graves were shallow pits, sub-rectangular in shape, with the bottoms of the graves resting on bedrock and the tops covered by paving slabs (Weber et al. 2007). Two-thirds of graves registered use of fire, to some extent, while roughly one-third (17 graves) showed varying degrees of charring on skeletal elements (Weber et al. 2007).

## **1.5 Chapter Summary**

In summary, the Cis-Baikal was inhabited by two biologically distinct cultures, the EN Kitoi culture and the LN-EBA ISG cultural complex, separated temporally by a period of cemetery burial cessation. Archaeological investigation has shown that the EN Kitoi had a high reliance on fishing resources, but also hunted terrestrial game. Populations were unevenly distributed across the landscape and displayed cultural heterogeneity, with adult males likely in the most dominant positions (Link 1999; Weber et al. 2002; McKenzie 2003; Weber and Bettinger 2010). Residential mobility was low seasonally, with base camps tied to major waterways. Males engaged in more extensive/logistical travel for resources unavailable at the home base and had heavier workloads over time. EN individuals also appeared to experience high levels of physiological stress at times (Weber and Bettinger 2010:Table 6). On the other hand, LN-EBA ISG populations utilized terrestrial game and aquatic resources more equally. Population sizes were more evenly distributed across the landscape and social differentiation between sexes was less evident within groups. Workloads appear to have been less than in the EN and less logistical foraging is thought to have taken place. Overall physiological stress appears to have decreased from the EN (Weber and Bettinger 2010:Table 6). Little is known about plants in the diet of either culture. Both cultures would have been consuming a very high protein/low carbohydrate diet based on dietary studies to date.

This study seeks to analyze dental calculus in Cis-Baikal populations from both the EN and LN-EBA in order to support and add data on the diet of these groups, including the possible role of plants, and to suggest ways that these groups may have used their mouths as tools. Four large, well-documented cemeteries were chosen for examination: Lokomotiv and Shamanka II representing the EN Kitoi, and Ust'-Ida I and Khuzhir-Nuge XIV representing the LN-EBA ISG. These sites are located within different microregions of the Cis-Baikal area. Lokomotiv and Ust'-Ida I are both located within the Angara River Valley microregion, while Shamanka II and Khuzhir-Nuge XIV are both situated on the shores of Lake Baikal, within the South Baikal and Little Sea microregions respectively. The next chapter discusses the multicausal nature of dental calculus formation and how it can be used to obtain information about an individuals' life histories, diets, environments, genetic backgrounds, etc. The characteristics of the environments and cultures discussed above are among the many factors that have the potential to influence calculus formation and therefore need to be considered in efforts to analyze and interpret these deposits.

## **Chapter 2**

### **Dental Calculus – Composition, Aetiology, and Archaeological Use**

Dental calculus can provide a wealth of information about past lifeways through analysis of its presence and severity, as well as through observations of particles embedded within its matrix. This chapter provides a detailed outline of the composition and aetiology of calculus to illuminate its multicausal nature and its potential usefulness in an archaeological context. Past research has used calculus abundance to interpret the proportions of dietary components, specifically proteins and carbohydrates, while new research has begun to focus on the microscopic analysis of the deposits themselves. As plaque begins to mineralize into calculus, bacteria and debris from food and artifacts processed or manipulated with the teeth can become preserved in the matrix, providing direct evidence of items consumed or utilized in the mouth (Blatt et al. 2010). The theories and methods outlined here are used specifically on individuals selected from the four cemeteries described in the previous chapter to determine the utility of dental calculus for dietary and mouth use reconstruction in the Cis-Baikal region of Siberia.

#### **2.1 Plaque Formation**

Dental calculus is mineralized plaque that adheres to tooth surfaces (Hillson 1996). The analysis of dental calculus therefore begins with a detailed look at the circumstances under which dental plaque forms. Dental plaque exists as a biofilm within the oral cavity. A biofilm is a complex, communal, three-dimensional arrangement of bacteria within a polysaccharide extracellular matrix (Gurenlian 2007). The biological basis for many dental diseases is largely a consequence of the composition of this complex dental microbial flora (Hillson 2005:286).

Plaque biofilms develop through a number of stages, from pioneer to climax communities. It should be noted that, although these stages are listed here as separate events, these events are known to overlap because plaque formation is a dynamic and continuous process (Marsh and Bradshaw 1995) which does not result in a uniform structure for every case. It varies from tooth to tooth and from location to location within an individual (Rosan and Lamont 2000). The first stage involves the formation of the acquired pellicle, which is a condensed layer consisting mainly of salivary or gingival crevice fluid (GCF) components, including proteins and

peptides, which are deposited onto the tooth surface (Marsh 2010). This pellicle layer makes the tooth surface receptive to bacterial colonization by providing attachment sites to cells and occurs within minutes after eruption or cleaning (Clerehugh et al. 2009; Gurenlian 2007; Marsh 2010; Rosan and Lamont 2000). During early colonization, pioneer organisms, mainly aerobic Gram-positive streptococci bacteria, become weakly attached to the pellicle in a reversible stage of adhesion. Next, short-range stereochemical interactions between the pioneer microbial cells and receptors on the pellicle result in more permanent adhesion (Clerehugh et al. 2009:Table 4.1). Rapid growth occurs, as adherent bacteria secrete a large amount of water-insoluble extracellular polysaccharides which contribute to form a biofilm matrix, and microcolonies grow within this matrix (Gurenlian 2007). The matrix contains components derived from saliva or GCF as well (Hillson 2005; Marsh and Bradshaw 1995). Secondary colonizers begin to adhere to earlier pioneer species in a process known as co-aggregation, and structural stratification within the plaque is established (Clerehugh et al. 2009:Table 4.1; Gurenlian 2007; Marsh 2010). Attached bacteria multiply, increasing the size of the plaque deposit (Clerehugh et al. 2009:Table 4.1), and generally hundreds of diverse species of bacteria exist together within the resulting plaque deposit (Rosan and Lamont 2000). Eventually a steady state/equilibrium is reached. Interior bacteria become static and slow their growth rates; the cells become disrupted and often devoid of cell contents making them susceptible to mineralization. Surface bacteria remain active (Gurenlian 2007). The climax community which has developed at this point commonly contains high levels of Gram-negative anaerobic filamentous organisms, many of which are associated with dental diseases (Marsh 2010).

Saliva provides beneficial buffering to changes in the oral cavity and has antimicrobial properties to protect the enamel of the teeth (Marsh and Bradshaw 1995). As plaque accumulates, it creates an environment that is protected from saliva; the development of a biofilm protects the bacteria living within it. It generates its own plaque fluid, which bathes the bacteria and their associated matrix, forming the interface between the oral environment and the plaque microcolonies. Its chemistry is important in influencing certain dental diseases such as carious lesions, periodontitis, and dental calculus (Hillson 2005).

## **2.2 Dental Calculus Formation**

Dental calculus formation is always preceded by plaque formation, with the plaque accumulations forming an organic matrix which is then subject to the subsequent mineralization

processes that create calculus (Clerehugh et al. 2009; Roberts-Harry and Clerehugh 2000). The presence and activity of bacteria are very important; almost no calculus deposits are formed in the absence of bacteria (Hazen 1995; Mandel 1990). Calculus formation is quite complex and a matter of debate, but at its simplest, it occurs due to the deposition of calcium and phosphate salts within the bacterial plaques (Mandel 1990).

There are two forms of dental calculus, supra- and subgingival, based on their location on the tooth in relation to the gingival (gum) line (Roberts-Harry and Clerehugh 2000; White 1997). Supragingival calculus accumulates above the gum line and is most frequently found on tooth surfaces in close proximity to salivary glands, specifically the lingual sides of the anterior mandibular teeth or the buccal sides of the maxillary molars, particularly the first molar (Clerehugh et al. 2009:Table 6.1; Mandel 1990). Salivary secretions, which diffuse into the plaque fluid, are the main source of mineral salts in supragingival calculus (Mandel 1990). Subgingival calculus accumulates within the gingival pocket, below the gum line. It seems to have no preferential location of development within the oral cavity and the primary source of mineral salts comes from GCF rather than saliva (Clerehugh et al. 2009:Table 6.1). Formation seems to occur in a similar pattern for both types of calculus (Mandel 1990). Therefore, the formation process discussed here will apply broadly to both, but there are significant differences between the two including morphology, composition, mineral content, and appearance (see Clerehugh et al. 2009:Table 6.1 for a summary). For example, subgingival calculus is considered to have a slightly darker brown colour and to be more dense and compact, with higher ratios of calcium to phosphate, when compared to supragingival calculus (Clerehugh et al. 2009:Table 6.1; Little and Hazen 1964). Most differences can only be seen microscopically (Lieverse 1999).

The formation of calculus begins with the stages of plaque formation defined above (formation of the pellicle, attachment, growth, etc.). About five days after the initial pellicle formation, the plaque has been replaced by mostly filamentous bacteria and the matrix resembles mature decalcified calculus (Mandel 1990). The plaque absorbs calcium and phosphate from saliva or GCF, and an alkaline pH within plaque fluid is considered to be critical in promoting mineralization through crystallization of these components (Sissons et al. 1988; Wong et al. 2002; Jin and Yip 2002). The crystal growth associated with mineralization is initiated at localized sites, usually in the interbacterial plaque matrix, spreads around the walls of the bacteria, and then finally extends within the bacterial cells themselves (Mandel 1990; White

1997; Zander et al. 1960). Plaque covers the calculus surface in life, creating new matrix for secondary/continued mineralization. Secondary/continued mineralization makes use of the existing mineralized periphery for its base (Schroeder 1969). Dental calculus is an age-progressive condition (Duckworth and Huntington 2005; White 1997; Anerud 1991); therefore secondary mineralization will continue to enlarge deposits if they are not removed (e.g. due to oral hygiene practices).

The inorganic portion of calculus is composed mostly of calcium phosphate, which exists in a variety of structural forms within deposits. Hydroxyapatite is the most common structural form but octocalcium phosphate, whitlockite, and brushite also occur in varying quantities (Grøn et al. 1967; Hazen 1995; Jin and Yip 2002; Lieverse 1999; Tan et al. 2003). Other structural forms exist but are rare (Grøn et al. 1967). Ratios of each structural form depend on the stage of maturity of the deposit. Brushite and octocalcium phosphate precipitate out of solution quickly and therefore are more abundant in immature deposits while hydroxyapatite and whitlockite use these as precursors and become more abundant as the deposit matures (Driessens and Verbeeck 1989; Mandel 1990; Lieverse 1999). Magnesium, sodium, potassium, lead, and other trace elements are also found within the inorganic component of calculus (Hazen 1995).

Organic components comprise about 15 to 20 percent of the dry weight of a deposit (Mandel 1990). This includes amino acids, peptides, glycoproteins, carbohydrates, and lipids (Mandel 1990; Hillson 1996; Lieverse 1999). Proteins and carbohydrates are derived primarily from entombed bacteria and from saliva (Mandel 1990). Also, channels of organic matrix sometimes containing non-mineralized bacteria often extend into the calculus (Tan et al. 2004).

There are many theories that attempt to explain the mechanisms behind this mineralization process. Mandel (1990) summarizes the main theories. The Booster Mechanism Theory suggests that mineralization begins when local pH, calcium, and phosphate levels reach high enough levels to allow the precipitation of calcium phosphate salts. Elevation of pH can occur if there is a loss of carbon dioxide in the saliva/GCF, or if ammonia is being produced in the oral cavity by bacterial metabolism of amino acids or urea (Mandel 1990; Margolis 1990; Schroeder 1969). This theory is now thought to be too simplistic (Lieverse 1999).

The Epitactic Concept Theory is more widely supported (Lieverse 1999), and suggests that the calcium and phosphate concentrations in tissue fluids and saliva are not high enough to cause mineralization on their own but form a metastatic solution that can support crystal growth



once it begins (Mandel 1990). Nucleation, the development of an initial crystal “seed,” requires a certain organic matrix that will provide the exact structural configuration needed for the first hydroxyapatite crystal (Mandel 1990). The exact circumstances that result in nucleation are not well understood, but it requires more energy and a higher saturation of calcium and phosphate than is required for continued crystal growth after nucleation has occurred. The metastatic solution will therefore support growth from thereon in. (Schroeder 1969).

The Inhibition Theory hypothesizes that mineralization can only occur in the absence of mineralization inhibitors (Mandel 1990). The enzyme alkaline pyrophosphatase is one such inhibitor that prevents the initial nucleation event. Oral bacteria are important in destroying inhibiting factors. They increase the thickness of plaque, increasing the barrier from inhibitors in saliva; they also secrete enzymes, such as protease, to degrade inhibitors (Soder 1972; Tetevoossian and Newbrun 1979 in Duckworth and Huntington 2006).

The Transformation Theory is based on the fact that hydroxyapatite need not immediately arise upon nucleation, as discussed above. Brushite and octocalcium phosphate can precipitate out of solution quickly, reducing the threshold needed for nucleation. These can be used as precursors to form whitlockite and hydroxyapatite through transformations over time (Driessens and Verbeeck 1989; Mandel 1990).

Mandel (1990) suggests that all of these theories may help to explain part of the calcification mechanism, but there is such variability in formation that any one theory does not adequately explain all aspects of the process.

### **2.3 Aetiology of Dental Calculus**

The aetiology behind plaque and calculus formation processes is multicausal (Lieverse 1999), which explains the high level of variability between deposits. Figure 2.1 provides a chart to help demonstrate the complexity of this system. Many of the factors, discussed below, affect the plaque accumulations directly, creating an environment potentially conducive to calculus formation; other factors affect calculus directly. Factors that directly influence calculus formation can either affect the nucleation or the growth of mineral crystals, while factors that influence plaque will affect either the microorganisms or the plaque fluid and matrix. While a multitude of factors can cause calculus to form on the tooth surface, it is quite difficult to remove. Deliberate or accidental mechanical removal is the primary means through which it can become detached (Kamath and Nayak 2014). In modern times this is done predominately

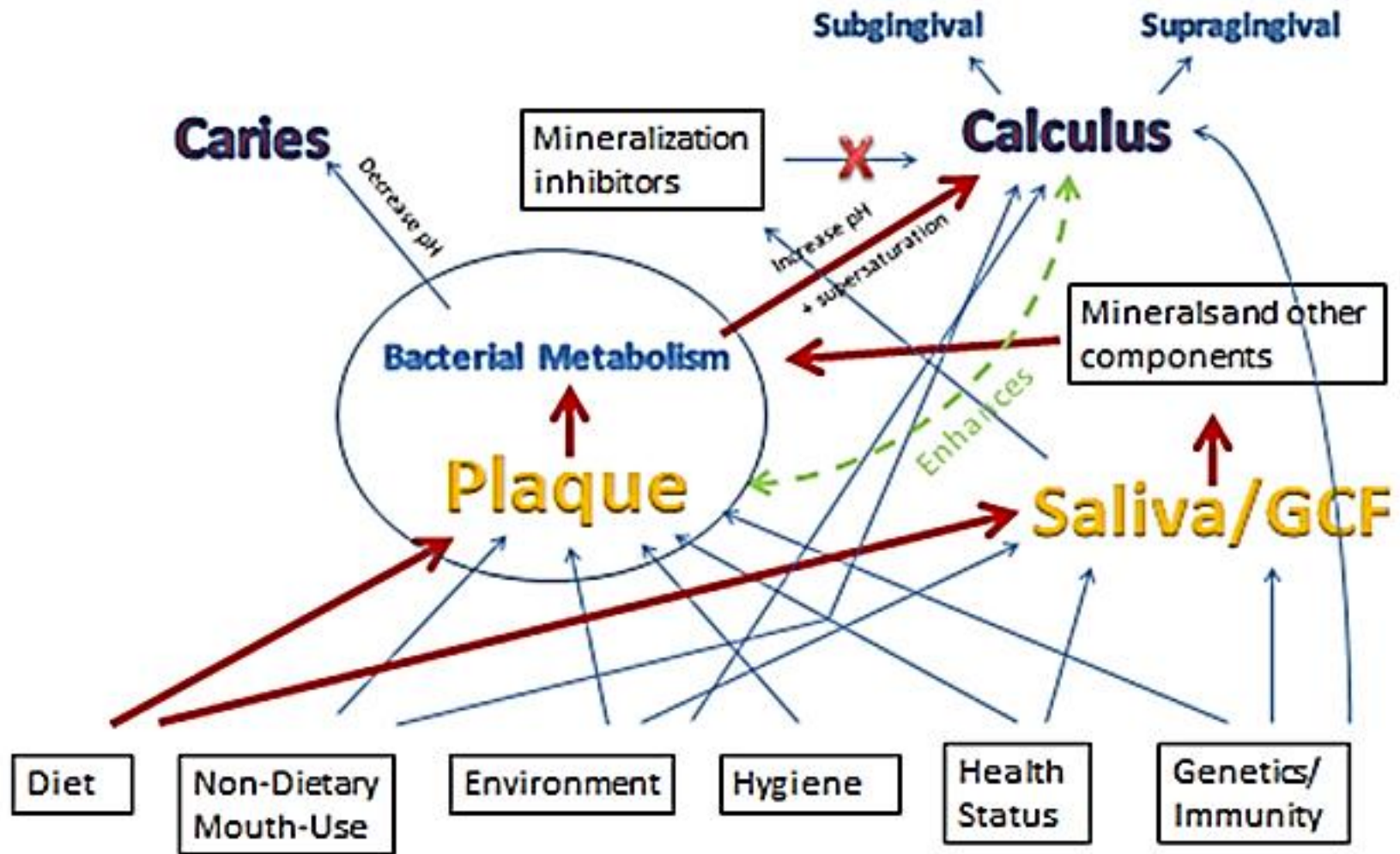


Figure 2.1 Factors (depicted in boxes along bottom) influencing plaque, calculus, and caries formation.

through clinical dental scalings (Kamath and Nayak 2014) but other types of abrasion, such as attrition from using the mouth as a tool or dietary grit causing dental wear, also have the potential to mechanically remove deposits (Clarke and Cameron 1998; Gaare et al. 1989; Hillson 2005; Lieveise 1999).

### *2.3.1 Dietary Factors*

An important factor that can influence plaque and calculus formation is diet. The best evidence that is available for dietary effects on calculus comes from studies on protein. It is generally accepted that a localized increase in pH in plaque is the main reason for calculus formation because it facilitates the precipitation of salts (Duckworth and Huntington 2006; Roberts-Harry and Clerehugh 2000; Wong et al. 2002). Dawes (1970) found that urea, a by-product of protein breakdown, was the only dietary component that conclusively affected the composition of saliva. The level of urea in saliva is directly related to the level of urea in the blood, which is directly related to protein intake (Nikiforuk et al. 1956 and Addis et al. 1947 in Dawes 1970). Urea from saliva is broken down, then diffuses through the plaque fluid and is metabolized by the microorganisms (Hillson 2005:288). Metabolism by-products alter the environment within the plaque. Sugar metabolism causes acid waste products, while amino acid and urea metabolism produces alkaline waste products (Hillson 2005:288). Hillson (2005:288) argues that the pH of plaque fluid at least partially reflects the balance of nutrients that are available to the bacteria. In other words, the pH of plaque can differ with the proportion of protein to carbohydrates in the diet. Increased protein will cause increased alkalinity in the plaque, which will result in more mineral ions precipitating out of solution, causing crystallization on the tooth surface (Hillson 1979; Jin and Yip 2002).

Increased carbohydrates will cause increased acidity in the plaque, resulting in the opposite, that is, demineralization on the tooth surface and the eventual formation of a carious lesion (Hillson 2005:291; Figure 2.1). A carious lesion is an infectious microbiologic disease of the teeth that results in localized dissolution and destruction of the calcified tissues (Roberson 2006). Dental caries will not be a focus for this research project, but its aetiology is important because it is strongly affected by diet and can provide useful archaeological information when compared with dental calculus deposits. Carious lesions are not caused by a single factor, but of importance is the presence and interaction of dental plaque bacteria and carbohydrates within the oral cavity (Ismail et al. 2013; Roberson 2006). If the proper combination of plaque bacteria and

carbohydrates, especially sucrose, occurs, the bacteria will metabolize the sugars to produce acidic waste products, decreasing the pH of the plaque substrate for a period of time (Kidd 2005). The acidic environment promotes the growth of disease-causing bacteria such as *Streptococcus mutans* and *Lactobacillus acidophilus*, which will then further increase the acidity of plaque through increased metabolism of carbohydrates. Repeated falls in pH result in the demineralization of dental tissues, progressively destroying the tooth surface and eventually forming a carious lesion (Ismail et al. 2013; Kidd 2005; Roberson 2006; Figure 2.1).

Researchers' understanding of bacterial metabolism establishes the idea of two polar outcomes, caries versus calculus, depending on the proportion of carbohydrates and protein in the diet. Yet microorganisms use carbohydrates, particularly sucrose, to synthesize polysaccharides which will increase the plaque matrix (Hillson 2005:288). More carbohydrates in the diet will result in larger and thicker plaque deposits, which bring about more opportunities for calculus formation. Therefore some literature also states that increased carbohydrate consumption results in increased calculus deposits after the biofilm has developed (Keenleyside 2008; Lieveise 1999; Lukacs 1989; Plumbo et al. 1963).

In recent years, attention has turned to the identification of oral-health-promoting foods or food components in contemporary humans. Studies show that some natural components in food and beverages can have an inhibiting effect on plaque formation by decreasing the adhesiveness of, or by acting against, oral bacteria (Signoretto et al. 2010; Weiss et al. 2004; Wu 2009; Yoo et al. 2011). Types of food and drinks that have been identified for their potential inhibitory effects on biofilm development include tea, raisins, wine, coffee, honey, cranberries, dried plums, and grape seeds (Signoretto et al. 2010; Weiss et al. 2004; Wu 2009; Yoo et al. 2011). Drinking and/or eating habits can thus affect the microbial community within plaque (Signoretto et al. 2010). If the natural progression of adhesion and activity of microorganisms is interrupted, then the potential for calculus formation will likely be affected as well.

Another aspect of diet that seems to have an effect on the formation of calculus is the presence of silicon. Research has shown that silicon can enhance the nucleation and growth of seeded crystals (Damen and ten Cate 1989; Hayashizaki et al. 2008; Rolla et al. 1989; White 1997). High levels of silicon are present in foods such as rice. Gaare et al. (1989) found higher rates of calculus formation in Asian populations and attributed this to the high proportion of rice in their diets. Through SEM-EDX analysis, Power et al. (2014) observed increased silicon

concentrations next to identifiable starch grains embedded in calculus. This suggests that dietary intake of silica or silicic acid enhances calculus formation, allowing for the better preservation of starch.

### *2.3.2. Non-Dietary Factors*

Figure 2.1 shows that diet is only one of many factors influencing calculus rates, and it may not even be a predominant factor (Lieverse 1999). Salivary and GCF factors that influence mineralization include mineral ion levels, proteins, and lipids (White 1997). These may vary between individuals due to diet, but also genetic individual variation, saliva and GCF flow rates, biological affinity, etc. (Dawes 1970; Francis and Briner 1969; Mandel 1973; Roberts-Harry and Clerehugh 2000; White 1997). Ions within the saliva or GCF diffuse into the plaque fluid (Hillson 2005). Calculus formation is directly associated with elevated calcium and phosphate levels in the blood and body fluids (Dawes 1970). Phosphate levels rise with calcium levels. Yet calcium in the blood and saliva is very closely regulated by the body, and therefore is likely affected very little by changes in dietary calcium (Dawes 1970). Differences are then largely based on non-dietary factors. Some studies on animals have suggested that higher levels of calcium and phosphates in the diet are important for increased calculus formation (Francis and Briner 1969, König 1968), but the elevated calcium and phosphate levels of experimental diets in these studies may be unrealistic in relation to typical human dietary variation. Mandel (1973) found that in the insoluble portion of plaque, increased calcium and phosphorus levels, and decreased potassium and carbohydrates clearly distinguished heavy calculus formers from light calculus formers. Regardless of where these inorganic minerals are originating, after reviewing the literature available, Duckworth and Huntington (2006) concluded that a direct relationship between increased levels of both calcium and inorganic phosphate and calculus does exist, though it is not always statistically significant. The influence of other components is less certain.

Another significant factor in plaque and calculus formation is oral hygiene. Oral hygiene plays a large role in the amount of plaque that accumulates on a tooth surface (Marsh and Bradshaw 1995). White (1997) suggests that it is very important to split populations into two groups when studying calculus development. Accumulations develop very differently in populations that engage in the regular removal of plaque and calculus deposits as opposed to those in which accumulations are allowed to progress along their natural course. Populations with regular oral hygiene generally have supragingival calculus deposits confined around the

mandibular lingual surfaces of the anterior teeth, next to the submandibular salivary gland, and the maxillary buccal molars, next to the parotid salivary glands (White 1997). Subgingival calculus formation is more widespread within the oral cavity. Both types of deposits typically begin to form in the teenage years, but do not increase significantly with age due to regular removal of deposits. In populations that do not practice regular hygiene, both supra- and subgingival calculus deposits occur in almost 100 percent of cases and are widespread throughout the dentition (White 1997). This is supported by a well-known study of the natural progression of calculus in Sri Lankan tea labourers, where, by 40 years of age, all individuals had calculus on almost every tooth (Anerud et al. 1991).

Mouth use and environment should also be considered as factors in the formation of plaque and calculus. Any situation where gritty materials enter the oral cavity may have the potential to actively remove deposits. This hypothesis has been applied to abrasive diets (Clarke and Cameron 1998; Gaare et al. 1989; Hillson 2005) and contexts where the teeth are used as tools (Lieverse 1999), but can be extended to include any grit from the environment that may come in contact with the teeth. Drinking water is another environmental factor that should be noted here. Hard or soft water may contribute ions and minerals to saliva that will have an effect on plaque and calculus (Gaare et al. 1989; Damen and ten Cate 1989; Lieverse 1999). For example, silicon, already discussed for its dietary effects on calculus, can also occur as an ion in water and become incorporated in saliva or GCF and affect calculus formation rates (Damen and ten Cate 1989).

The Inhibition Theory suggests that a number of mineralization inhibitors are present in saliva, and that these need to be removed for calculus to form. Inhibitors can be present in either the saliva or GCF, and can either affect nucleation or crystal growth (Clerehugh et al. 2009). Known inhibitors include salivary pyrophosphate, salivary proteins, and magnesium (Clerehugh et al. 2009; Duckworth and Huntington 2006). These will not be discussed in any detail here, but it is important for a researcher to know that they exist within the oral cavity or substances introduced to the oral cavity and may influence the quantity of calculus that develops.

Some people can be considered light or heavy calculus formers, regardless of diet or hygiene. It is likely that a genetic/individual factor is responsible for this phenomenon. Support for this comes from studies such as one by Mandel (1973), where differences in mineral content in plaque were evident for predetermined heavy and light calculus formers. Each was asked to

desist in all hygiene activities over the course of the study. No diseases were noted for any individual. Regulation of many minerals is controlled by the body (e.g., Dawes 1970), and therefore is likely influenced by genetic/individual variation, moderating or limiting other factors, such as diet.

Studies have confirmed genetic and population distinctions in regards to calculus formation (Christersson et al. 1992; Roberts-Harry and Clerehugh 2000), and the physiological stress levels of an individual may be important as well (Arensburg 1996; Hazen 1995; Martins et al. 2012). Physiological imbalances and stress may play a greater role in oral conditions and calculus accumulations than originally thought. Based on a review of non-human primate studies, Arensburg (1996) concluded that captivity-related stress and physiological deficiencies were related to incidences of oral pathological conditions. In all, researchers need to understand that diet is but one factor in many that influences dental health, and these other factors cannot be ignored when attempting to analyze calculus deposits.

## **2.4 Using Extent and Location of Calculus to Study Diet**

Since it is mineralized, dental calculus survives well archaeologically and is often used during bioarchaeological analysis to attempt to determine dietary patterns (Lieverse 1999). However, as established in the previous sections, diet is not the only factor that contributes to calculus formation. The red arrows in Figure 2.1 illustrate the pathways of interest if we are to extrapolate diet from calculus. However, there are many other factors that potentially impinge on these pathways and therefore may obscure our ability to determine dietary effects on calculus formation, and these need to be considered when conducting research. A number of case studies, summarized here, help to highlight how the extent and location of calculus in past populations has been used to provide useful information on past dietary patterns, and also to help illuminate some of the issues regarding interpretations.

The logic behind using extent and location of calculus to determine aspects of diet is that these variables can be assessed and recorded across multiple individuals to determine frequencies based on the presence/absence of dental calculus, or to calculate indices based on its severity (e.g., Brothwell 1981; Greene et al. 2005; Hillson 1979). Comparisons among populations or subgroups of a population should then indicate any differences in the type of diet most commonly eaten by those groups, assuming, of course, that systematic variations in extent and location of calculus are to some significant degree controlled by dietary factors in the

populations being investigated (Hillson 1979). These approaches to the study of dental calculus are well-documented, and a standard procedure has been established by Brothwell (1981). This involves scoring the amount of calculus on individual teeth as absent (0), small (1), moderate (2), or large (3). Location is recorded, as well, and sometimes multiple tooth surfaces are scored (e.g., Greene et al. 2005). Numerous types of statistical analysis can then be performed depending upon the research question.

Hillson (1979) conducted a study on skeletal material from Egyptian and Nubian sites, ranging from the Predynastic period to the Christian era, to try to reconstruct temporal and regional dietary patterns from the frequencies of oral pathological lesions present. Pathological data were presented as percentages of individuals affected by a given disease. It was found that caries rates were around 10 percent and calculus rates were around 50 percent. In contrast, a modern survey conducted in 1946 showed that 90 percent of sampled individuals from Cairo, Egypt, had some evidence of caries, while calculus and periodontal disease were almost universal. The high calculus and periodontal disease rates in the 1946 Cairo population are attributed to diets incorporating large amounts of vegetable foods with high protein and carbohydrate content. The caries rates are attributed to regular sugar intake. Hillson (1979) notes that the presence of fewer plaque-related diseases in ancient times reflects less extensive plaque deposits on teeth, yet that calculus rates were still high. This suggests that vegetables were a staple, as in modern times. However, the low caries rates suggest that sugar intake was low. This also explains the lower plaque accumulations. Pre-Dynastic samples had even lower frequencies of plaque-related diseases and may represent even lower sugar consumption or perhaps more meat in the diet.

Mesolithic and Neolithic hunter-fisher-gatherers in the Ukraine provide another situation where the calculus-to-caries relationship has been used to interpret diet (Lillie 1996). This study found that carious lesions were universally absent across the entire sample. However, a wide range of calculus deposition was evident within the sample population. Lillie argues that, since the typical age-progressive patterning of caries was not seen within this sample, one is led to conclude that dietary factors were of primary significance. The presence of calculus deposits is therefore attributed to higher rates of protein intake (e.g., a meat/fish/nut-based diet), while the absence of caries indicates low sugar consumption. Since this trend is seen in both the Mesolithic and Neolithic samples, they conclude that a hunter-fisher-gatherer lifestyle was present in both



periods, although other lines of evidence also detect a shift to pastoralism in the late Mesolithic to Neolithic periods.

Similar caries-to-calculus ratios were observed in the previous research conducted by Lieverse et al. (2007a) in the Cis-Baikal area of eastern Siberia, but the interpretation is quite different. They collected dental calculus data from two sites in the Cis-Baikal: Shamanka II (EN) and Khuzhir-Nuge XIV (LN-EBA). Materials from Lokomotiv (EN) and Ust'-Ida I (LN-EBA) were excluded from this original analysis due to evidence that dental calculus may have been removed from tooth surfaces during post-excavation cleaning and analysis, potentially biasing the data. Dental pathological lesions in the two cultures were analyzed to attempt to distinguish similarities and differences. Carious lesions were almost non-existent in both populations. Only four lesions were documented in total (Lieverse et al. 2007a). It was found that individuals from Shamanka II exhibited significantly higher calculus frequencies than individuals from Khuzhir-Nuge XIV for 13 of 32 teeth. Although Lillie (1996) argues in his study that the caries and calculus rates primarily reflect diet, Lieverse and her colleagues downplay the importance of diet because previous research supports that both the EN Kitoi and the LN-EBA ISG consumed similar diets characterized by low carbohydrates and high protein (Haverkort et al. 2008; Katzenberg et al. 2009; Katzenberg et al. 2012; Weber and Bettinger 2010). Instead they propose that the differences observed between the two populations are likely non-dietary. Individual variation may have played a role, but it is suggested that local environmental factors may have been a primary factor. This was because, as was mentioned in the previous chapter, the EN Kitoi are known to have had lower residential mobility and would have been more susceptible to the effects of local factors, such as differing mineral content in water sources (Lieverse et al. 2007a). This present study seeks to expand on this research by including data from Lokomotiv and Ust'-Ida I to test if calculus trends in additional samples align with dietary, environmental, and/or mouth use activity patterns. However, caution is taken with the data from Lokomotiv and Ust'-Ida I due to the aforementioned concerns regarding removal of calculus from specimens.

A number of studies also advocate for a positive relationship between calculus and carbohydrates. The Dental Pathology Profile (DPP) is a useful tool that attempts to correlate dental pathology frequencies with pre-agricultural and agricultural subsistence systems (Lukacs 1989). It is based on pathology data collected by Cohen and Armelagos (1984). Within the DPP, carious lesion frequency progresses from low to high as populations move from hunter-gatherer

to agricultural strategies. This makes sense, since agricultural diets incorporate more carbohydrates, allowing more plaque to accumulate and offering more opportunities for an acidic plaque environment. However, the DDP describes the same pattern for calculus frequencies. Frequencies go from low to high as groups move from hunter-gatherers to agriculturalists (Lukacs 1989). This is likely due to the proposed relationship between increased carbohydrate intake and increased plaque deposits. The polarity, or mutual exclusiveness, of carious lesions and calculus is no longer apparent within this model. This idea can also be seen in the work done by Keenleyside (2008) at the Greek colonial site of Apollonia. She found that carious lesion rates were moderate and trended to the lower end of the range typical of agricultural populations, being within the range of a mixed economy. Calculus accumulations were not large, but they occurred at high frequencies across the population, and she concluded that this is consistent with that of other agricultural populations, pointing to a reliance on a high carbohydrate diet and/or poor oral hygiene (Keenleyside 2008). Stable isotope data supported this conclusion.

When ancient microorganisms such as bacteria were discovered in calculus, Arensburg (1996) took this as evidence that oral pathological lesions related to diet are not just a disease of civilization. It is a popular belief that oral pathological lesions increase in populations from the Neolithic onward due to dietary shifts toward agriculture (Hillson 1979). This is not always supported by dental pathological evidence, though. The Natufian culture existed before the advent of agriculture in Israel, yet they exhibit similar pathological conditions and frequencies to cultures in the Neolithic, despite having had very different subsistence economies (Arensburg 1996). It is suggested that factors such as physiological health and stress should be considered in addition to diet as a cause for these frequencies.

This is only a small subset of the many studies that exist on the application of calculus frequencies to studies on ancient dietary reconstruction, but these case studies represent the wide variability and ad hoc explanation that occurs when interpreting the data in different contexts. Some interpret the data on a protein-carbohydrate spectrum, some on a spectrum of increasing plaque accumulations, some use a bit of both, and others look to factors beyond diet. It seems that often other factors are only considered if dietary evidence does not match up with calculus frequencies, and this should not be the case. Researchers need to be vigilant to ensure that they are aware of the many factors that contribute to calculus formation, and that these are taken into account during analysis to avoid biases. The strength in this approach is clearly seen in the case

of the Cis-Baikal, where previous work had established that diets in the study populations were more similar than not, allowing an informed consideration of how other factors may have generated the observed differences in calculus formation. Also, almost none of the articles discuss the possibility of heavy versus light calculus formers (see Section 2.3.2). This is something that would be challenging to see archaeologically, except perhaps through genetic analysis, but it has the potential to significantly skew interpretations. For instance, in the Cis-Baikal, there is a possibility that Khuzhir-Nuge XIV individuals were simply genetically prone to developing less calculus.

This thesis research does not assume that any one factor is the dominant factor in calculus formation. It seeks to examine the multiple factors that can be associated with calculus formation and compare it to the results of the study to conclude whether calculus severity provides a useful proxy for diet in the Cis-Baikal, or whether other factors more accurately help to explain the outcome. While it can be quite difficult to pinpoint these factors, here, significant correlations between calculus formation and established data from past research in the Cis-Baikal on diet, environment, mouth use activities, biological affinity, etc. provides good evidence for causative factors.

## **2.5 Plant Particles in Dental Calculus**

### *2.5.1 Significance*

New techniques for analyzing calculus are becoming increasingly popular as a means of expanding its interpretative value and reliability. These techniques generally involve the removal of calculus samples from the tooth surface and analysis of components based on microscopic identification of inclusions, stable isotope analysis, or mass spectrometry. Only the microscopic analysis of dental calculus will be discussed for this research project. While this may be seen as destructive compared to frequency analysis, dental calculus is generally considered a secondary skeletal remain, meaning that it is not considered an inherent part of the skeleton or dentition. Therefore, it is not technically considered destructive to the skeleton (Scott and Poulson 2012). Furthermore, these methods avoid many of the pitfalls of the previous studies discussed because they involve the direct observation of dietary components within the calculus deposits.

Microscopic analysis of inclusions in calculus is a well-known and established technique. As plaque begins to mineralize into calculus, bacteria and debris from food and artifacts

processed or manipulated with the teeth can become preserved in the matrix (Blatt et al. 2010). Salivary amylase protects the embedded particles from chemical breakdown (Mickleburgh and Pagan-Jimenez 2012). The inclusion of bacteria and other debris in the matrix provides direct evidence of what the population was consuming or utilizing, since the mineralization process requires the presence of saliva and therefore only takes place in vivo (Blatt et al. 2010). Calculus builds up on the tooth surfaces over an individual's life if not removed, so we may infer that at least several years of diet are represented in every individual studied (Piperno and Dillehay 2009).

The typical procedure involves scraping deposits off of teeth, weighing the samples, and mounting them onto slides for analysis under a microscope. Calculus samples are usually prepared for mounting by one of two methods: either the sample is simply crushed into a powder and placed directly onto a slide (e.g., Henry 2012; Piperno and Dillehay 2008) or the calculus is chemically broken down, centrifuged, and the demineralized product is mounted (e.g., Hardy et al. 2009; Li et al. 2010; Wesolowski et al. 2010; Blatt et al. 2010).

### *2.5.2 Microparticles*

The microscopic identification of plant particles in archaeological contexts has enhanced the recovery of data pertaining to human and plant relationships in the past (Pearsall 2000). Two of the most common types of plant particles found within dental calculus are starch grains and phytoliths. These two particle types will be the focus of this study but other particles such as diatoms, spores, pollen, fibers, etc. will also be noted when present.

Phytoliths are particles of hydrated silica, absorbed from groundwater and redeposited in living plant cells (Piperno 2006). The silica is precipitated into intracellular or extracellular locations within the plant; therefore phytoliths usually take the shape of plant cells (Piperno 2006; Figure 2.2). Figure 2.2g provides a depiction of how phytoliths derive from the cells of plant tissue. The biological functions of phytoliths continue to be a subject of debate, but, among other things, they add support and rigidity to some plant parts (Piperno 2006). They occur generally, but not exclusively, in the less edible portions of the plant including stems, leaves, roots, and inflorescences (Pearsall 2000). They are liberated from other less durable organic plant tissues after they die and decay (Piperno 2006). Phytoliths are produced in many plant species, often being identifiable to particular genera, and they exhibit remarkable durability in soils and sediments over long periods of time (Pearsall 2000; Piperno 2006). They have the

ability to provide information about diet, the palaeoenvironment, or items manipulated with the teeth. Past studies have often documented only low phytolith counts from calculus deposits (Lalueza Fox and Pérez-Pérez 1994; Lalueza Fox et al.1996; Henry and Piperno 2008), but in some cases, they have helped to provide significant insight into past diet or the environment in which an individual lived (e.g., Dudgeon and Tromp 2012).

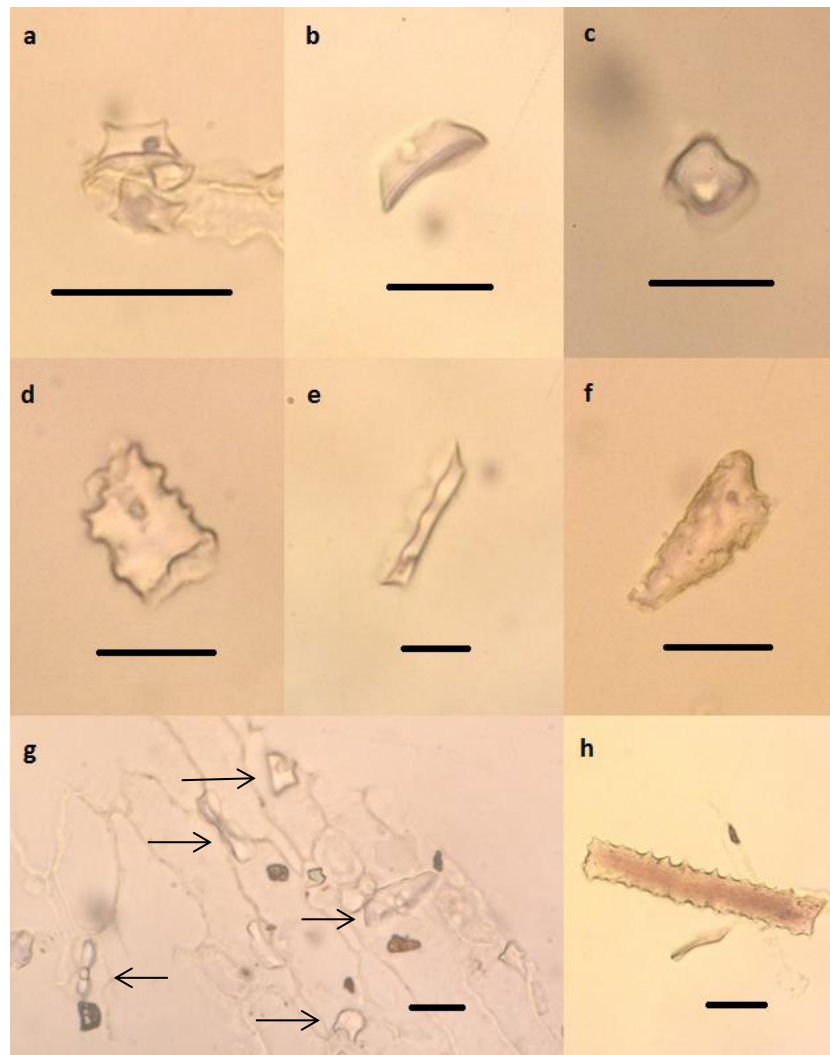


Figure 2.2 Various phytoliths from modern soil cores from the Red Tail Site, Wanuskewin Heritage Park (courtesy of Lauren Stead), scale= 15  $\mu$ m.

Starch, in contrast, is produced in different parts of the plant as a form of energy storage. Glucose, formed by photosynthesis, can be converted into transient starch within chloroplasts or can be transported into specialized units known as amyloplasts, where is it converted into

storage/reserve starch (Haslam 2004). Transient starch grains are very small, ranging from 0.2 to 7  $\mu\text{m}$ , with an indeterminate shape (Haslam 2004). They are produced during the day when photosynthesis rates are high and are either used as energy or converted to reserve starch at night (Gott et al. 2006). Reserve starch grains come in many shapes and sizes. They are generally between 1  $\mu\text{m}$  and 100  $\mu\text{m}$  and are designed for long term energy storage (Figure 2.3). Starch can be found in almost every kind of plant tissue, but reserve starch is largely concentrated in storage organs such as roots, tubers, fruits, and seeds, which humans and other animals exploit as food sources (Gott et al. 2006). Like phytoliths, some reserve starch granules are quite diagnostic and can be identified to specific plant families, genera, or species, making them very useful for dietary reconstruction. Transient starch grains are generally smaller than reserve starch grains, less readily identified microscopically, and lack diagnostic features; therefore they are not thought to be identifiable to plant taxa (Gott et al. 2006).

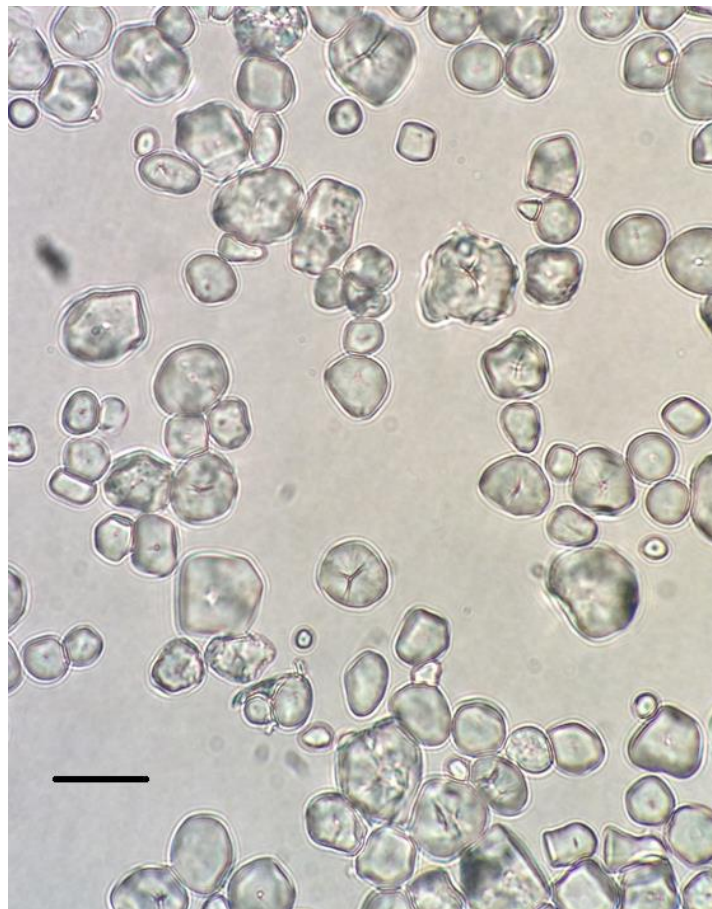


Figure 2.3 Modern reserve *Zea mays* starch grains from comparative slide, scale= 20  $\mu\text{m}$ .

Other particles that are known to occur in dental calculus include diatoms, fibers, spores, and pollen. These are all generally not incorporated into calculus through diet, but are introduced to the oral cavity through other processes such as non-dietary mouth use and environmental exposure. Diatoms are single-celled algae typically encased in a cell wall made of intricately laced silica (Gross 2012). Various species are found in distinct ocean and freshwater environments, as well as soils, making them useful in palaeoenvironmental reconstruction (Dudgeon and Tromp 2010; Power et al. 2014), particularly as their siliceous walls preserve well archaeologically (Gross 2012). They can become embedded within calculus when they are introduced to the oral cavity through media such as drinking water. Fibers such as cellulose/cotton or other vegetative fibers have been identified within calculus in the past (e.g., Charlier et al. 2010; Blatt et al. 2010). These would have potentially become embedded within calculus from chewing or non-dietary mouth use. It is also possible that meat fibers may be present, but no such identification has been published to date. Spores are reproductive cells used by plant species such as ferns, fern allies, moss, liverworts, and lichen (Sandiford 2012). Pollen grains are the reproductive male gametes of flowering plants (Leuschner 1993). Both have outer walls that are organic but extremely resistant to physical and chemical breakdown (Leuschner 1993; Sandiford 2012). In most instances spores and pollen would likely have become embedded within calculus through the environment (e.g., suspended in air or water), contamination in food, or non-dietary tool use. Both are rarely reported and, when they are mentioned as an inclusion in calculus, they are rarely used to contribute information on cultural dynamics (e.g., Charlier et al. 2010; Power et al. 2014). These may represent aspects of the environment in which past populations lived, but they may also represent adherent contaminants from the burial environment.

Many research projects in the past have been quite successful in the recovery of plant particles from calculus samples of varying antiquity. Starch grains have been observed in samples at least 40,000 years old (Henry et al. 2010), while phytoliths are known to have been preserved within calculus for millions of years (Henry 2012; Henry et al. 2012). A brief look at some previous research helps to highlight the circumstances in which calculus has yielded such particles, showing the usefulness of this approach for dietary reconstruction.

First, the microscopic analysis of particularly ancient dental calculus has helped to shed light on the diets of ancient hominoids. Henry et al. (2012) recovered a variety of phytoliths,

including examples representing fruit, grass, wood/bark, and leaves, from calculus samples taken from an *Australopithecus sediba* specimen dated to approximately two million years ago. This provides evidence that *Au. sediba* likely had a varied diet. Dental calculus samples from five Neanderthal individuals from El Sidrón Cave in Spain (between 46,000 to 36,000 years B.P.) were analyzed by Hardy et al. (2012), and a number of starch granules were observed in nine of ten samples analyzed. This suggests that these Neanderthal individuals included starchy foods in their diet. Also, a study conducted on dental calculus from a *Gigantopithecus blacki* individual, dated to one million years B.P., yielded phytoliths from both fruits and grasses (Ciochon et al. 1990).

Henry and Piperno (2008) conducted an analysis of far more recent calculus from five modern *Homo sapiens* individuals from Tell al-Saqā'i, Syria. Dating roughly to the third millennia B.C., the site is considered to have been used for cereal storage and processing, so the researchers were quite surprised when the ample starch grains extracted from the calculus included only a few grains representing domesticated barley and none representing evidence of wheat. However this actually supports archaeological evidence that the inhabitants of the site were engaged in a specialized economic activity of storing cereals for trade, and were not typically consuming the cereal grains themselves. Instead it appears they were relying on a variety of wild and cultivated foods for their own consumption, although most of the starch grains recovered were unidentifiable at the time of analysis.

Dudgeon and Tromp (2010) analyzed calculus samples from 104 individuals from Rapa Nui (Easter Island) from the late sixteenth to early eighteenth century A.D. and observed 4,733 phytoliths and 11,644 diatoms. The phytolith assemblage was dominated by globular echinate morphotypes, consistent with an extinct palm that existed on the island prehistorically. Sugarcane, considered to be a food staple of Polynesia, represented only a minor part of the assemblage (5.85 percent). Previous studies suggested that the palms were almost completely removed from the island prior to the late prehistoric period to which the skeletons are dated. This suggests two possibilities: 1) the obsidian-hydration dates associated with the burials need to be reassessed, or 2) the deforestation of the island was completed at a later time than previously thought. The latter possibility is significant because it not only alters the interpretation of diet during this time period but also possibly provides new palaeoenvironmental data on the deforestation event. High frequencies of diatoms recovered from individuals from the southern



parts of the island also suggest a separate source of drinking water than individuals from other areas.

A study on calculus samples from pre-Columbian insular Caribbean individuals dating from ca. 350 B.C. to A.D. 1,600 found that maize was more commonly consumed in the area than originally thought, based on counts of observable starch grains (Mickelburgh and Pagán-Jiménez 2012). Damage to the starch grains was also used to suggest that the maize was ground, baked, and consumed as bread. Other starch grains identified suggest a broad spectrum, but locally available diet.

Blatt et al. (2010) analyzed 18 calculus deposits from the Danbury site in Ohio and identified fibers consistent with cotton from within a Late Woodland component, using scanning electron microscopy. Prehistoric cotton had not been previously documented in Ohio and its discovery here provides evidence for long distance trade with groups in the American Southwest. The fibers likely were incorporated into the oral cavity while processing raw fibers. This attests of the ability for microscopic dental calculus analysis to provide information beyond dietary inclusions.

Many studies have used this technique to look at the diets of animals as well. Middleton and Rovner (1994) used barnyard herbivores from an American Colonial period site to determine that phytolith assemblages found in dental calculus provide useful data on these animals' diets, allowing reconstruction of livestock management practices and environmental change. Hardy et al. (2009) analyzed calculus from two modern deceased chimpanzees to demonstrate the potential for large quantities of starch grains to be preserved within the deposits. Finally, phytoliths from the calculus of four American mastodons reflected major dietary components and suggest that they consumed more grasses than were previously assumed, given that they are traditionally considered browsers rather than grazers (Gobetz and Bozarth 2001).

Again, this is only a small sample of the studies that have successfully extracted plant particles from dental calculus, but it highlights their utility as direct lines of evidence for what individuals were eating or processing with the mouth, as well as the potential of this approach to provide palaeoenvironmental data.

## **2.6 Chapter Summary and Research Objectives**

In summary, dental calculus formation is complex and multicausal. Factors that are known to influence plaque and calculus formation include diet, non-dietary mouth use, hygiene,

genetics, physiological stress, and environment. As factors interact and influence formation, the specific influence of any one factor becomes obscured. All possible factors need to be considered to tease out the reality of what dental calculus severity is representing with regards to ancient lifeways. Chapter one highlighted the similarities and differences between the EN Kitoi and LN-EBA ISG Cis-Baikal cultures. Prominent dietary patterns, differences in physiological stress levels, levels of social differentiation, genetic affiliation, mobility patterns and microregional differences in the environment are all well-established through past BHAP research and can be useful when analyzing the calculus severity patterns for these populations; correlations may suggest causative factors. In contrast, past research shows the value of microscopic analysis to study particles within calculus deposits that were in direct contact with the oral cavity during the life of the individual. This avoids the issues of a multifactorial system obscuring dietary influences. It can shed light on plants utilized, dietary or otherwise, water sources, and the palaeoenvironment.

There are two parts to this research project. The first is to examine the severity of dental calculus from EN and LN-EBA groups in the Cis-Baikal to determine similarities and differences within and among the populations. If diet ratios of protein to carbohydrates are a significant factor, high rates of calculus formation are expected for individuals within each of the cemetery populations; there should be little to no significant differences among individuals due to the evidence of a high protein/low carbohydrate diet provided through stable isotope analysis and frequencies of other dental pathological lesions (Lieverse et al. 2007a; Katzenberg and Weber 1999; Weber et al. 2011; Weber and Bettinger 2010). This component of the research is designed around three research questions:

1. Are there significant differences in calculus deposition among sex and age groups within each of the cemetery populations?
2. Are there significant differences in calculus deposition among the cemetery populations?
3. When comparing the results of the first two questions with previously obtained stable isotope and dental pathology results, is dental calculus a good proxy for diet and/or mouth use in the Cis-Baikal? If not, based on the results, what other factors may be affecting calculus formation?

The second part of this project is a pilot study that involves the direct microscopic observation of plant particles embedded within calculus deposits to determine if, and to what extent, plants were included in the diet of each cemetery group. Little research has been conducted on plants in the diets of middle Holocene Cis-Baikal populations to date. There are two research questions:

1. Are plant particles visible within the dental calculus of individuals from the Cis-Baikal area? If so, do these particles have the potential to yield useful information about Cis-Baikal hunter-gatherer lifeways?
2. Is this a feasible area of research for future projects? Further, do opportunities for new information on plants and other particles in contact with the oral cavity outweigh any limitations?

## **Chapter 3**

### **Methodology**

As indicated in Chapter 2, this research project involves the study of dental calculus based on two methods: a macroscopic analysis of the severity of dental calculus and a microscopic analysis of plant particles embedded within dental calculus. The macroscopic analysis is used to determine whether dietary trends and other factors known to affect dental calculus formation can be assessed among Cis-Baikal populations. The microscopic analysis is used to determine if plant microfossils can be recovered from samples of calculus material from the Cis-Baikal and whether these microfossils can provide data on plants that have been in contact with the oral cavity.

#### **3.1 Macroscopic Analysis**

All dental calculus macroscopic observations were conducted in a laboratory, housed at the Irkutsk State University, containing all of the Cis-Baikal material included within this study. This research focused only on four of the largest and most well-documented cemeteries: Lokomotiv, Shamanka II, Ust'-Ida I, and Khuzhir-Nuge XIV.

##### *3.1.1 Data Collection*

Every observable permanent tooth was scored for the quantity of dental calculus, according to slightly adapted versions of the standards outlined by Greene et al. (2005) and Brothwell (1981). It was presumed that most deciduous teeth would not have calculus deposits therefore they were not included in analyses. Greene et al. (2005) took the scoring system most commonly used in calculus research, developed by Brothwell (1981), and converted it to develop calculus indices to better look at severity. Brothwell's (1981) system consisted of giving a tooth a score of 0, 1, 2, or 3, representing no deposit, small deposit, moderate deposit, or large deposit respectively (Table 3.1 and Figure 3.1). Greene et al. (2005) used this scoring system but divided the tooth into three surfaces (buccal, labial, and interproximal) so that scores were assigned to each surface rather than the whole tooth. For this analysis, the Greene et al. (2005) method was used but the interproximal surface was further divided into the mesial and distal interproximal surfaces, so that each interproximal surface could be looked at individually. Therefore each tooth

was separated into four surfaces, buccal/labial, lingual, mesial interproximal, and distal interproximal, and each surface was given a score to record the amount of calculus on that surface.

Table 3.1 Dental Calculus Scoring System

Score	Details
0	No calculus present
1	Less than 1/3 of tooth surface covered in calculus
2	Between 1/3 and 2/3 of tooth surface covered in calculus
3	More than 2/3 of tooth surface covered in calculus
9	Unobservable surface

Adapted from Brothwell 1981 and Greene et al. 2005

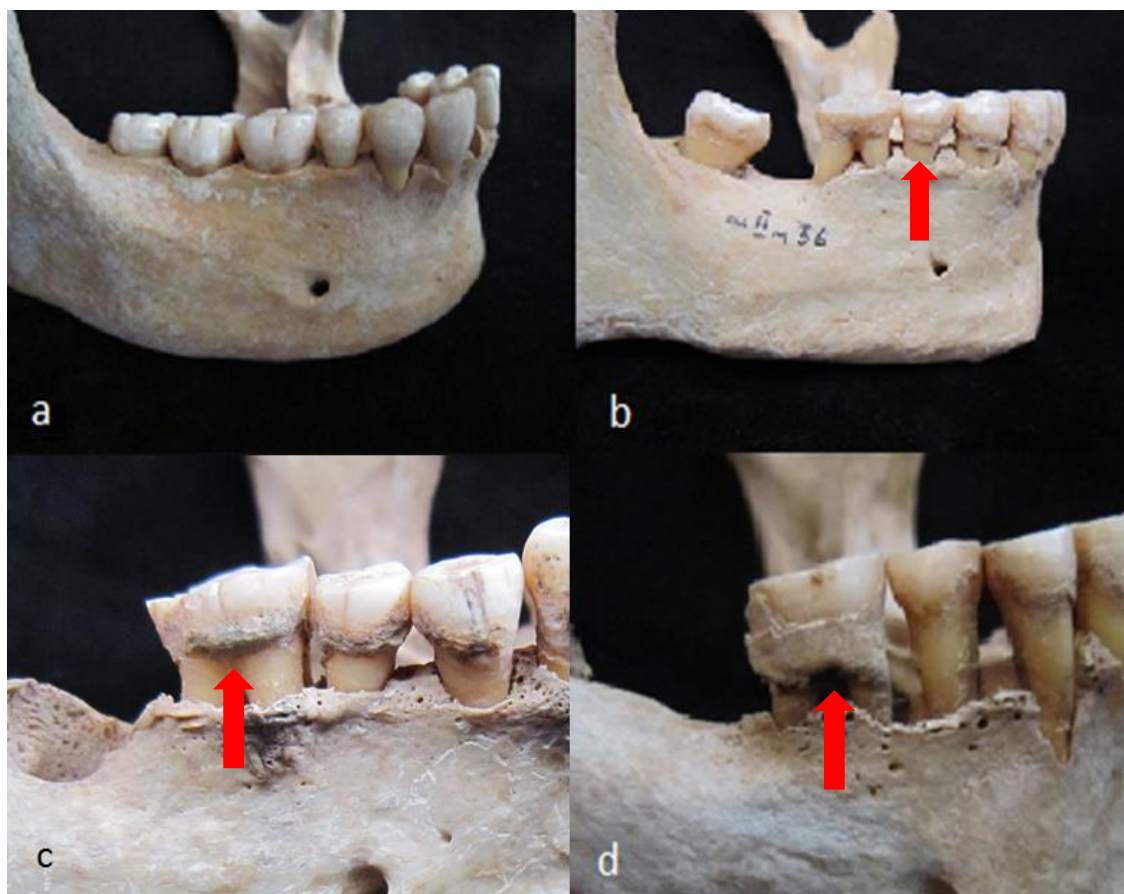


Figure 3.1 Examples of dental calculus deposit scores from Cis-Baikal individuals (deposits indicated by red arrows): (a) Score of 0, Shamanka II Burial 11-1; (b) Score of 1, Shamanka II Burial 36; (c) Score of 2, Shamanka II Burial 46-1; (d) Score of 3, Shamanka II Burial 30-1.

A tooth was considered recordable if it was a fully erupted permanent tooth with at least one observable surface. A surface was considered observable if there was no evidence of postmortem damage and if the view was unobstructed by resin, non-calculus mineral deposits, or another adjacent tooth tightly in occlusion, with neither tooth removable from their sockets. Antemortem attrition was not treated the same as postmortem breakage. Previous research stated that many of the teeth from the Cis-Baikal populations exhibited moderate to severe dental wear (Lieverse et al. 2007a). It was decided that calculus on heavily worn teeth would be scored as if it were an estimated average sized tooth crown surface for the population being studied. This prevented high scores for worn surfaces due to their reduced surface area. A number of teeth exhibited attrition so severe that no crown remained. It was decided in the field that, unless the surface exhibited postmortem breakage, it was considered observable regardless of the amount of wear. The reasoning behind this was that calculus can form on the root surface as readily as on the enamel surface, and calculus did occur on the roots in many cases from these populations; therefore there should be no reason to value the enamel surface over the root surface. Consequences of this decision are discussed in the following chapter. Deposits on roots, as well as large deposits extending over both crown and roots, were scored as if they occurred on an estimated average sized crown surface as well.

Raw data on all surfaces scored for calculus deposits are provided in Appendix A, Table A-1. An inventory of every tooth, along with the calculus scores applied to it, was done according to the standards outlined by Buikstra and Ubelaker (1994:49; Table 3.2) so as to keep track of observable teeth, but also to provide more information about unobservable teeth (e.g., extensively damaged teeth, undeveloped teeth, antemortem loss, and postmortem loss) (Appendix A, Table A-1).

The calculus surface scores were used to develop indices to provide a measure of the severity of calculus formation (Greene et al. 2005). Greene et al. (2005) found that calculus indices, separated by quadrant, were more effective at showing trends and differences among and within cemetery populations than frequency analysis. Lieverse et al. (2007a) conducted a presence/absence study based on dental calculus frequency, the results of which are described in Chapter 2, Section 2.4, on individual teeth from Shamanka II and Khuzhir-Nuge XIV individuals. Rather than just elaborating on this research by adding information from Lokomotiv

Table 3.2 Scoring System for Inventory of Dentitions

Score	Details
1	Present, not in occlusion
2	Present, development complete and in occlusion
3	Missing, no associated alveolar bone
4	Missing, with alveolus resorbing or fully resorbed, ante-mortem loss
5	Missing, with no alveolar resorption; post-mortem loss
6	Missing, congenital absence
7	Present, no measurements due to damage
8	Present, but unobservable (e.g., deciduous or permanent tooth in crypt)

From Buikstra and Ubelaker 1994: 49; Number scores are converted into a colour-coded system within Appendix A, Table A-1

and Ust’-Ida I, the use of calculus indices was chosen to attempt to discover new trends and patterns from the data available.

Indices were developed for four quadrants within the mouth (upper anterior [UA], upper posterior [UP], lower anterior [LA], and lower posterior [LP]), following Greene et al. (2005). Note that upper and lower refers to the maxillary and mandibular regions of the mouth respectively. Greene et al. (2005) separated the mouth into quadrants, rather than creating one index for the whole mouth because individuals had greater representation of surfaces in some areas of the mouth than others. Separating into quadrants allowed for the elimination of poorly represented areas and retention of well represented areas (Greene et al. 2005). The division of the mouth into these four quadrants also reflects tooth function. Chewing and grinding of food occur in posterior teeth while anterior teeth tear and slice food (Martini et al. 2012). These functional differences would likely effect plaque and calculus accumulations. In addition, main salivary ducts occur in the UP and LA quadrants (Chapter 2, Section 2.2; Mandel 1990). Together, the separation of the four quadrants offers a more detailed and dynamic study of dental calculus since calculus does not form uniformly throughout the mouth. To calculate an index, the scores for each observable surface in a quadrant were summed and divided by the total number of surfaces scored (Greene et al. 2005). A calculus index for the UA quadrant included the combined scores for the maxillary incisors and canines divided by 24 (six teeth multiplied by four surfaces), if all teeth were present and all surfaces observable (Greene et al. 2005). Indices calculated for each individual in the Cis-Baikal are summarized in Appendix A, Table A-2.

Although an individual may have had observable teeth/surfaces, it did not mean that they were included in subsequent statistical analysis. There were a large number of teeth lost both

postmortem and antemortem from many individuals in each of the cemetery populations. For example, during data collection it became apparent that many mandibular molars were missing from individuals from each of the cemeteries. After inquiring into past research, it was found that either the right or left first, second, and third mandibular molars had been removed for stable isotope analysis (e.g., Haverkort et al. 2008, Katzenberg et al. 2012). While the information that has been obtained from stable isotope analysis has been very important and valuable, the loss of these teeth was unfortunate with regards to this analysis. It drastically decreased the number of observable surfaces in the LP quadrant of the mouth.

An index based on only one or two surfaces would obviously not be representative of the quadrant. To solve this problem it was decided that at least 30 percent of the permanent tooth surfaces present *at the time of death* needed to be observable for that quadrant to be included in the analysis (Appendix A, Table A-2). The quadrant represented the individual in each analysis. Therefore, if an individual had 52, 65, 25, 10 percent representation for the UA, UP, LA, and LP quadrants respectively, the individual would be included in UA and UP analyses but not for mandibular quadrants. Only counting permanent teeth that would be present at the time of death meant that more juveniles could be included in analyses, since deciduous tooth surfaces would not count towards the overall total of surfaces that should be present. Ideally, at least 50 percent of the tooth surfaces in the quadrant would be present to be included in the analysis, as in the study by Whittaker et al. (1998), but studies have used lower percentages with good results. Arnay-de-la-Rosa et al. (2009) required eight teeth to be present to be included in the analysis (i.e., minimum of 25 percent of surfaces had to be present). Thirty percent fell within this range, while also helping to keep sample size as large as possible.

In total, 197 Cis-Baikal individuals had enough observable tooth surfaces to obtain a calculus severity index for at least one quadrant: 46 individuals from Lokomotiv (EN), 81 individuals from Shamanka II (EN), 34 individuals from Ust'-Ida I (LN-EBA) and 36 individuals for Khuzhir-Nuge XIV (LN-EBA) (Table 3.3). Only burials dating to the dominant cultural period were included in analyses.



Table 3.3 Complete List of Individuals Included in the Analysis of Dental Calculus Severity

<b>Shamanka II</b>											
<b>Burial</b>	<b>Age</b>	<b>Sex</b>	<b>Burial</b>	<b>Age</b>	<b>Sex</b>	<b>Burial</b>	<b>Age</b>	<b>Sex</b>	<b>Burial</b>	<b>Age</b>	<b>Sex</b>
6	16-18	PM	7	20-30	PF	8-1	35-40	M	10	25-35	M
11-1	18-20	F	11-2	30-40	M	12	20-35	*	13-3	18-19	PF
14-1	25-30	M	14-2	20-25	F	15	25-35	M	17-1	30-40	M
19	25-30	M	21-1	25-30	M	21-2	25-30	M	21-3	16-18	U
22	19-22	M	23-1	35-45	PM	24-1	25-35	M	26-3	6-8	U
29-1	20-30	M	30	35-50	M	32	35-45	M	33	35-45	M
34	35-45	M	35-1	25-35	M	36	25-35	PM	39	40-44	M
41	30-39	M	42-1	40-45	F	44-1	50+	PM	45	25-35	M
46	25-29	M	47	20-25	F	48-1	50+	M	49-1	17-20	PM
50-1	25-35	M	50-2	25-29	M	51	20-25	M	53-1	20-25	M
54-1	17-21	F	55-1	35-39	M	55-2	5-7	U	56-2	8-10	U
57-1	25-29	F	57-2	25-35	F	58-1	35-45	M	59-1	35-39	M
59-2	15-19	PF	60-2	40-44	F	61-1	25-29	F	61-2	35-45	M
62-5	45-59	M	63-1	25-29	M	63-2	25-35	M	64-1	30-39	M
65	50+	M	66-1	25-35	F	67	7-9	U	69-1	25-30	F
69-2	20-25	F	70	40-50	M	71	35-45	M	73	16-18	F
74	18-20	M	75	25-29	M	76	40-50	M	77	30-39	F
78-1	16-18	F	78-2	25-35	F	78-3	20-25	M	79-1	35-50	PF
85	25-35	M	88	6-8	U	90	18-20	M	92	10-12	U
93-2	35-40	F	96-2	30-35	F	104	25-35	F	108-1	35-50	M
108-3	25-35	M									
<b>Lokomotiv</b>											
<b>Burial</b>	<b>Age</b>	<b>Sex</b>	<b>Burial</b>	<b>Age</b>	<b>Sex</b>	<b>Burial</b>	<b>Age</b>	<b>Sex</b>	<b>Burial</b>	<b>Age</b>	<b>Sex</b>
L 1-1-1	35-50	M	L 4-1-1	35-50	F	L 7-1-1	40-45	F	L 8-1-1	40-45	M
L 9-1-1	20+	F	L 10-1-1	20-25	M	L 10-2-1	20-25	M	L 11-1-1	50+	M
L 13-1-1	25-30	M	L 14-3-1	10-11	*	L 15-1-1	20-35	M	L 16-1-1	45-55	M
L 19-1-1	50+	M	L 20-1-1	20-29	F	L 20-2-1	35-50	M	L 21-1-1	50+	F
L 23-1-1	20-25	M	L 24-3-1	4-7	U	L 24-4-1	8-10	U	L 25-1-1	35-40	F
L 25-5-1	35-50	M	L 27-1-1	15-18	M	L 28-1-1	35-40	F	L 30-1-1	35-40	M
L 30-2-1	35-40	M	L 31-1-1	35-50	U	L 31-2-1	25-30	M	L 33-1-1	35-45	M
L 34-1-1	35-45	F	L 35-1-1	20+	U	L 37-1-1	25-29	F	L 38-2-1	35-45	F
L 39-1-1	20-25	F	L 41-1-1	15-20	F	L 41-2-1	5-7	U	L 42-1-1	40-50	M
L 43-2-1	20-29	F	R 2-1-1	35-50	M	R 7-1-1	50+	M	R 9-1	6-7	U
R 11-1	20-25	F	R 12-1	10-12	U	R 13-1	6-8	U	R 14-1	30-39	M
R 15-1	20-35	F	R 15-2	35-40	F						
<b>Ust'-Ida I</b>											
<b>Burial</b>	<b>Age</b>	<b>Sex</b>	<b>Burial</b>	<b>Age</b>	<b>Sex</b>	<b>Burial</b>	<b>Age</b>	<b>Sex</b>	<b>Burial</b>	<b>Age</b>	<b>Sex</b>
5-1-1	7-9	U	6-1-1	35-50	M	8-1-1	6-8	U	9-1-1	6-7	U

<b>Ust'-Ida I (continued)</b>											
<b>Burial</b>	<b>Age</b>	<b>Sex</b>	<b>Burial</b>	<b>Age</b>	<b>Sex</b>	<b>Burial</b>	<b>Age</b>	<b>Sex</b>	<b>Burial</b>	<b>Age</b>	<b>Sex</b>
10-1-1	9-11	U	11-1-1	35-50	F	12-1-1	35-50	M	14-1-1	18-20	M
16-2-1	25-35	M	18-1-1	11-13	U	19-1-1	30-35	M	20-1-1	18-24	M
20-2-1	30-40	F	21-2-1	5-7	U	22-1-1	15-20	F	24-1-1	14-18	F
25-2-1	7-9	U	25-3-1	9-11	U	26-1-1	13-15	U	26-4-1	10-12	U
26-5-1	5-7	U	29-1-1	50+	M	30-1-1	50+	F	31-1-1	10-12	U
32-1-1	8-10	U	33-1-1	12-15	U	38-1-1	35-45	M	44-1-1	9-10	U
44-3-1	11-12	U	45-1-1	22-30	M	48-1-1	30-40	M	53-1-1	9.5-11.5	U
56-1	35-50	M	56-2	9-11	U						
<b>Khuzhir-Nuge XIV</b>											
<b>Burial</b>	<b>Age</b>	<b>Sex</b>	<b>Burial</b>	<b>Age</b>	<b>Sex</b>	<b>Burial</b>	<b>Age</b>	<b>Sex</b>	<b>Burial</b>	<b>Age</b>	<b>Sex</b>
10	20-25	U	11	35-50	M	12	25-35	U	14	35-50	PM
15	25-35	M	16	7-9	U	17	5-7	U	19	35-50	F
27-1	35-50	M	27-2	9-11	U	27-3	4-6	U	32	50+	F
34	25-35	M	35-1	18-20	PM	35-2	8-10	U	36-1	35-50	U
37-1	14-17	U	37-2	14-17	U	38	35-50	M	39	9-11	U
44	35-50	M	45	8-10	U	46	25-35	M	48	7-9	U
50	15-18	U	51	18-20	M	53	35-50	M	55	35-50	PM
57-2	35-50	PM	58-1	25-35	U	58-2	35-50	PM	59-2	18-20	M
60	50+	PF	64	25-35	M	68	25-35	PM	77	12-15	U

Age and sex data from Lieverse, 2005; Age: years of age at death; Sex: M=male, PM= probable male, F= female, PM= probable female, U= undetermined sex

No distinction was made between sub- and supragingival calculus for this study because of the problems inherent when examining dry specimens. Supra- and subgingival calculus is distinguished by its location with regards to the gingival line. Without soft tissue as a reference, it can often be quite difficult to determine supra- and subgingival calculus based on location alone. Since periodontitis and continuous eruption due to high dental wear were known to have been prevalent in the Cis-Baikal populations (Lieverse et al. 2007a), it cannot be assumed that calculus present on the roots of teeth was subgingival during life. The roots may have been exposed above the gingival line due to alveolar resorption and thus the calculus may have been supragingival rather than subgingival. While it has been documented that there is a slight difference in the color of supra- versus subgingival calculus (Roberts-Harry and Clerehugh 2000; Clerehugh et al. 2009:Table 6.1), postmortem staining from soil, ochre, and bronze was noted on a number of Cis-Baikal remains, obscuring the natural colour of the deposits. Therefore this distinction could not be used.

### *3.1.2 Statistical Analysis*

Individuals were split into groups based on sex and age for statistical analysis. Age and sex determinations had been previously determined through BHAP research based on the recommendations of Buikstra and Ubelaker (1994). Age estimations relied mainly on changes to the pubic symphysis, iliac auricular surface, palatal and cranial sutures and, for subadults, on epiphyseal fusion and tooth eruption and development (Lieverse 2005). Sex determination was based on sexually differentiated characteristics of the pelvic bones and cranial features, with postcranial measurements also being used in some cases (Lieverse 2005). The most recent adjustments to age and sex estimates for each of the four cemeteries were conducted by A. R. Lieverse (Angela Lieverse, personal communication 2014).

Both Shamanka II and Khuzhir-Nuge XIV contained “probable” males and females, as well as more confidently identified males and females. For this analysis, probable males were compared with confidently identified males to determine if there were significant differences in calculus severity between the two groups. If they could be statistically determined to exhibit no significant difference in calculus scores from their more confidently identified counterparts, the two groups were pooled for all subsequent analyses. If significant differences were detected, the two groups could not be pooled and probable males were discarded from analysis. Sample sizes were too small to conduct this analysis on females and probable females from both Shamanka II and Khuzhir-Nuge XIV; hence no probable females were used in analyses. Any individuals that could not be identified to sex were grouped into an undetermined category. The age interval previously determined for each individual (see Table 3.3) was used to assign them to groups for juveniles, young adults, and older adults; these groups were assigned factor values of 1, 2 and 3 (Table 3.4; Appendix A, Table A-2). If an individual’s estimated age range overlapped with the dividing ages between two factors, they were put in the factor group that the range more fully overlapped. For example, if an individual was given an age range of 30-45 years of age based on Buikstra and Ubelaker (1994), they would be placed within factor 3 (older adults) because more of their age range overlapped with that factor.

Originally the calculus data were collected as ordinal scores (i.e., 0, 1, 2, and 3) but when converted to indices, they became essentially continuous variables. Since the goal of this research was to demonstrate whether or not the severity scores from each quadrant were

Table 3.4 Age Categories

Factor	Age	Group
1	<20 years	Juveniles
2	20-35 years	Young adults
3	>35 years	Older adults

significantly different among and within each of the four cemetery populations, a t-test or ANOVA analysis was appropriate. Initial data exploration showed that the data were not normally distributed (Appendix B, Figures B-1 to B-9). This may be due to the original categorical nature of the data. Heterogeneity of the data was also apparent; this was especially seen when comparing the variance of calculus scores between Ust'-Ida I and Shamanka II and juveniles versus adult groups (Appendix B, Figure B-10 to B-13). Log transformation of the data would have created more ideal distributions but it would have also eliminated zeros, which were important data on absence. Therefore, since the assumptions of parametric testing were violated, nonparametric Kruskal-Wallis and Mann-Whitney U tests were used instead of ANOVA and t-tests, respectively. Nonparametric tests measure medians rather than means which are then used to determine a rank for each cemetery. Some of the original data is lost in this process, making it less powerful than parametric testing but it is the most common way to deal with data that are not normally distributed and/or display heterogeneity of variance (Pallant 2010). These tests were performed to determine differences among different sex and age groups and also among the cemetery groups. Significance was determined when the p value was less than 0.05 ( $p < 0.05$ ). For Kruskal-Wallis tests, where more than two populations were compared, when significant differences were detected, post hoc testing was completed using stepwise step-down multiple correlation analysis to pinpoint exactly where differences were originating. Stepwise step-down analysis generated homogenous subset tables. Each population or group was placed on the table, ordered from lowest to highest, and arranged within subsets based on their level of significance to other groups (Nussbaum 2015). Population groups that appeared in the same subset were considered to be statistically similar, while groups that did not appear in the same subset were significantly different ( $p < 0.05$ ). A minimum of three individuals were needed to conduct any statistical analysis. All statistics were done using IBM SPSS Statistics (v. 22)®.

During data exploration, it became obvious that there were a number of outliers within the data set (Appendix B, Figure B-10 to B-13). Outliers, observations with very large or small

values compared to the majority of the observations, have the ability to significantly affect/skew results and need to be carefully watched for. At the same time, it is irresponsible for a researcher to remove an outlier simply because it is an outlier (Zuur et al. 2010). Outliers were referenced back to the raw data to make sure that the observation/measurement was taken accurately and correctly. Some individuals may have had scores slightly affected by limited representation of tooth surfaces (about 30 percent present and observable), but overall, many of the outliers were individuals with quite good representation of surfaces. Therefore, outliers were retained within the data set because there were no reasonable grounds for their removal. A hypothesis for why these outliers exist within this data set is presented in the following chapter.

Lastly, data exploration and knowledge of dental calculus formation indicated that juveniles and adults should not be combined within the same analysis. Plotting calculus severity for each quadrant versus age estimates, taken by computing the midpoint of the age range given for each individual, it can be seen that direct relationships were evident within this data set to varying extents (Appendix B, Figure B-14). This is significant because it suggests that a juvenile was more likely to have less calculus than an adult, a pattern consistent with the generally age progressive nature of dental calculus (Duckworth and Huntington 2005; White 1997; Anerud 1991). Grouping all ages together may have masked the effects of other variables. Also, because this study only included teeth that would have been present at death, developmentally incomplete teeth did not count towards the overall severity score. Juveniles could therefore have fewer overall surfaces present and still be included in the analysis and, because development was incomplete, the data was inherently different than for adults. Therefore, statistical tests did not compare juveniles to adults for both intra- and intersite analyses, although boxplots of the two groups were compared only to establish that the age-progressive nature of the deposits existed within the cemetery populations Lokomotiv and Ust'-Ida I.

### **3.2 Microscopic Analysis**

The primary purpose of the microscopic analysis portion of this thesis was to establish the feasibility of extracting and directly observing plant particles related to diet and mouth use from dental calculus samples collected from Cis-Baikal individuals. While the identification of plant particles to specific plant families or genera would have been an ideal outcome, there was no established comparative collection of starch grains or phytoliths for the Cis-Baikal region of Siberia. Consequently, identification was difficult or impossible for many of the particles, and it

was never the primary goal of this study. Detailed descriptions, as outlined below, and photographic documentation will hopefully aid in positive identification of species by future studies.

### *3.2.1 Contamination Protocol*

Previous research on plant particle recovery from ancient dental calculus immediately suggested that contamination with modern particles, especially starch grains, needed to be closely monitored (e.g., Dudgeon and Tromp 2012; Hardy et al. 2009; Li et al. 2010; Torrence 2006b; Wesolowski et al. 2010). Starch grains are commonly used in things like adhesives, paper, textile products, food, powdered gloves, and soaps (Zarrillo 2012; Torrence 2006b). It can easily become airborne and cause contamination (Torrence 2006b). The ideal protocol, to conduct all work within a positive pressure fume hood (e.g., Dudgeon and Tromp 2012; Wesolowski et al. 2010), was not an option available for this study. The protocol outlined here was largely based on that developed by Zarrillo (2012). Her protocol was designed to control contamination during the excavation, storage, and processing of residuals on artifacts such as stone tools and pottery, but it was also very applicable for this research project since her study involved similar non-ideal laboratory conditions. Based on a combination of strategies from previous dental calculus research (Dudgeon and Tromp 2012; Li et al. 2010; Wesolowski et al. 2010) and Zarrillo's (2012) dissertation, a contamination protocol was developed for both the Irkutsk State University lab, discussed in Section 3.2.2, and the University of Saskatchewan lab, discussed in Sections 3.2.3 and 3.2.4.

### *3.2.2 Data Collection in Irkutsk, Russian Federation*

All of the skeletal material analyzed as part of this research project was located in a small laboratory at the Irkutsk State University. Ten individuals per population were selected for microscopic analysis to locate in situ plant particles (Table 3.5). Selection was determined in the field and prioritized medium to large calculus accumulations; however, this emphasis was balanced against the need to include individuals from all age groups and both sexes, as well as samples from various locations within the oral cavity.

Development of a contamination protocol was very important since the skeletal and dental remains had been handled for years following their excavation and therefore the chance of previous contamination was high. A small workspace was isolated and designated for dental

Table 3.5 List of Individuals Sampled for Microscopic Analysis of Dental Calculus

SHA (EN)			LOK (EN)			UID (LN-EBA)			K14 (LN-EBA)		
Burial	Age	Sex	Burial	Age	Sex	Burial	Age	Sex	Burial	Age	Sex
66-1	25-35	F	L-20-2-1	35-50	M	56-1	35-50	M	32	50+	F
76-1	40-50	M	L-16-1-1	45-55	M	38-1-1	35-45	M	35-2	8-10	U
109-1	40-50	F	R-11-1	20-25	F	33-1-1	12-15	U	33	3-5	U
47-1	20-25	F	R-14-1	30-39	M	30-1-1	50+	F	11	35-50	M
30-1	35-50	M	L-35-1-1	20+	U	14-1-1	18-20	M	52	25-35	U
26-3	6-8	U	L-38-2-1	35-45	F	6-1	35-45	M	27-1	35-50	M
46	25-29	M	R-15-1	20-35	F	45-1-1	22-30	M	35-1	18-20	PM
60-2	40-44	F	L-28-1-1	35-40	F	44-2	5-6	U	34	25-35	M
24	25-35	M	L-15-1-1	20-35	M	19-1	30-35	M	37-2	14-17	U
67	7-9	U	L-9-1-1	20+	F	5-1-1	7-9	U	12	25-35	U

Age and sex data from Lieveise, 2005. Age: years of age at death; SHA= Shamanka II, LOK= Lokomotiv, UID= Ust'-Ida I, K14= Khuzhir Nuge XIV, Sex: M=male; PM=probable male; F=female; PF=probable female; U=undetermined sex.

calculus collection exclusively. The window located next to the isolated workspace was taped closed so plant particles could not enter the area via cracks in the window and the workspace surface was wiped down with a bleach solution after each use. Sterile powder-free gloves were used in the direct handling of any calculus material and were changed after taking each sample. No calculus deposits considered for sampling were contacted without gloves and other researchers in the lab were also instructed not to contact calculus deposits directly; this protocol did not, of course, mean that the potential risks of such contacts were known or avoided during past studies of these skeletal remains. All dental tools, tooth brushes, and swabbing brushes were autoclaved at the University of Saskatchewan and transported to Siberia in air-tight packaging to ensure that they were free from plant particles. No dental tool was used twice to ensure that no cross contamination of calculus samples occurred. However, some paintbrushes, used for swabbing skeleton and container surfaces only, were sterilized in the field due to diminishing sterile resources towards the end of the laboratory analysis (Appendix C, Table C-1). Sterilization in the field consisted of soaking the tool in undiluted bleach and rinsing with reverse osmosis (RO) water.

Deposits were cleaned with RO water and a tooth brush to remove any contaminants on their surfaces, then scraped off of the teeth with a dental tool either directly into a sterile microcentrifuge tube, if possible, or onto weigh paper first. Care was taken to ensure that the

tooth surface remained undamaged during this process (Figure 3.2), though in two cases enamel was so fragile that it crumbled with contact. Notes taken during sampling are provided in Appendix C, Table C-1. The samples were then transported back to the University of Saskatchewan for further analysis.

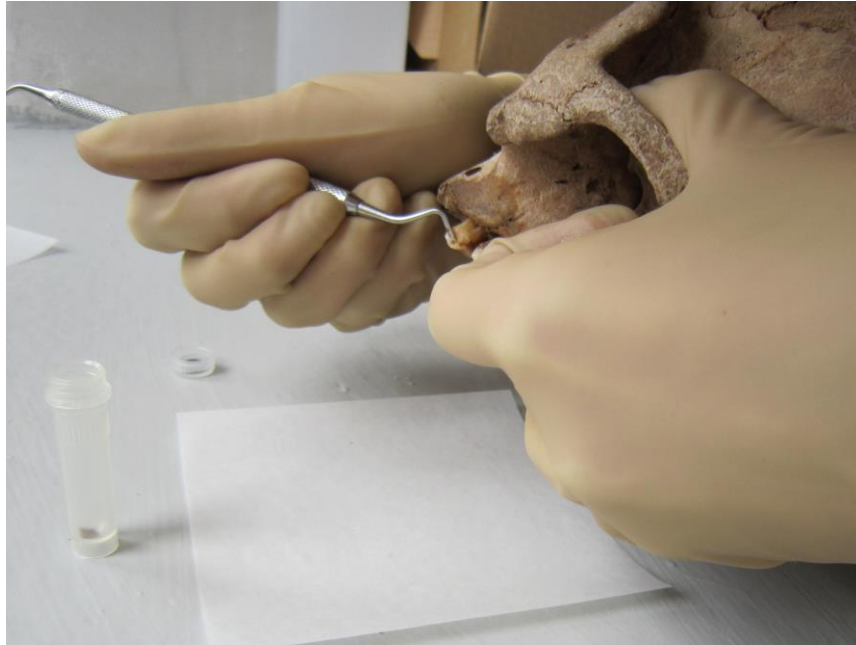


Figure 3.2 Removal of dental calculus sample from the tooth surface of Burial 109-1 from Shamanka II.

Since contamination could not be completely prevented, it needed to be monitored so that contemporary contaminants could be separated out from particles of ancient origin. This was done through the use of control swab samples. The insides of boxes, the counter, and other areas of the skeleton (e.g., mandible) were swabbed with sterile brushes and residues were transferred to microcentrifuge tubes with RO water. These were analyzed microscopically at the University of Saskatchewan lab for evidence of plant particles. A make-shift particle trap, consisting of immersion oil on a glass slide, was used to sample any airborne contaminants that could not be controlled by the measures implemented during sampling at the Irkutsk State University facility. The results of these controls are discussed in Chapter 4.



### 3.2.3 *Trials*

Trials were conducted to determine the optimal method to extract plant particles from the dental calculus. To undertake these trials, modern dental calculus samples were obtained from anonymous donors through the College of Dentistry at the University of Saskatchewan, with approval from the Biomedical Research Ethics Board (Bio #13-127, approved October 28, 2013). A review of recent studies on ancient dental calculus revealed that the most commonly used methods involved either physically crushing and mounting the samples, or chemically dissolving the matrix and mounting the residue. The latter approach typically involved demineralizing the samples with hydrochloric acid (HCl) solutions, although some studies combined this step with a second step involving deflocculation of the samples with sodium hexametaphosphate (NaHMP) (Blatt et al. 2010; Dudgeon and Tromp 2012; Hardy et al. 2009; Henry and Piperno 2008; Mickleburgh and Pagan-Jimenez 2012; Middleton and Rovner 1994; Li et al. 2010; Piperno 1988; Piperno and Dillehay 2008; Wesolowski et al. 2010).

There is debate over which approach is best. Some researchers insist that simply crushing and mounting the deposits to the slide is the best way to analyze particles in calculus because it guarantees that chemicals will not harm or alter particles with interpretive value (Piperno and Dillehay 2008). Yet, unless very well crushed, deeply embedded particles will likely not be released from the calculus with this method and many may remain unobservable. Thorough crushing also risks damage to starch grains and other particles due to grinding. Many researchers have turned to the use of acid, generally various concentrations of HCl, to remove the majority of the mineral matrix to make embedded particles easier to find (e.g., Henry and Piperno 2008; Li et al. 2010; Mickleburgh and Pagan-Jimenez 2012; Wesolowski et al. 2010). Acid can be damaging to organic particles such as starch, though, and care needs to be taken so that information that could have been obtained from dental calculus is not destroyed by the demineralization process. Henry and Piperno (2008) found that low concentrations of acid used over a 24-hour period did not damage starch grains. Many studies have also added a deflocculant like NaHMP; these are less harsh on certain particles, working to loosen adhered particles from the surface to ease dispersal and thereby improving microparticle recovery (e.g., Dudgeon and Tromp 2012; Henry and Piperno 2008; Li et al. 2010).

Combinations of these methods were applied to the modern calculus samples collected by the College of Dentistry but results were not as anticipated. A few plant particles were recovered,

but the samples did not dissolve with acid treatment and particles embedded deeper in the calculus were not released. Despite the risks posed by chemical damage, sample time in acid was increased from 24 to 48 hours in an attempt to encourage better dissolution but recovery rates did not improve. Wesolowski et al. (2010) stated that increasing time, rather than acidic concentration, was better for starch grain preservation. The limited success of this method may have been due to organic components in the calculus that were resistant to the physical or chemical methods used, allowing them to protect and hold the sample together even after processing. If so, it was anticipated that such components would be reduced or absent in archaeological specimens, as their organic content would have had an extended period over which to break down.

To test if the modern College of Dentistry samples were being held together by organic components after attempted dissolution and disaggregation, trials were ran on samples of greater age, where the organic components of the calculus had likely deteriorated. The Department of Archaeology and Anthropology at the University of Saskatchewan has a number of historic-era osteology teaching specimens with calculus deposits on the teeth. Six samples were taken to run three sets of trials, based on the three methods outlined below.

Trial one consisted of first weighing and crushing the sample, then rinsing with RO water and immersing it in an ultrasonic bath to loosen any contaminants on the surface. It was then centrifuged and the supernatant was pipetted off. A mounting medium consisting of 50 percent glycerol and RO water was added to create a roughly 0.20 mL sample and four to five drops (between 0.5 and 0.10 mL) were mounted on a slide under a coverslip with edges sealed using clear nail polish. Large coverslips were used (about four by two cm) to accommodate large subsamples.

Trial two consisted of weighing and crushing the sample, then rinsing it with RO water and placing it in an ultrasonic bath. It was then centrifuged and the supernatant pipetted off. Next, 1.5 mL of 10 percent HCl was added for 24 to 48 hours, agitating with a vortex mixer every few hours. The acid was then removed by centrifuging it, pipetting off the supernatant, and replacing it with RO water. This water wash was repeated twice. The sample was then dried out by removing the water via centrifuge again and replacing it with ethanol. The ethanol step was repeated twice, then the sample was left out to dry. An open centrifuge tube left out as a blank control beside the actual samples yielded four contaminant starch grains. Once dry, about 0.20

mL of 50 percent glycerol and RO water was added and the sample was re-suspended with a vortex mixer. Four to five drops (between 0.5 and 0.10 mL) were immediately transferred to a slide and sealed with clear nail polish as above.

Trial three consisted of weighing the sample, but not crushing it, then rinsing with RO water and placing it in a sonication bath. Next, 1.5 mL of 50 percent NaHMP and RO water were added to the sample and left for 24 hours, shaking often. After 24 hours, the deflocculent was removed with two water washes and replaced with 1.5 mL of 10 percent HCl for 24 to 48 hours. The trial then proceeded in a similar manner to trial two, except that after the two rounds of ethanol were added, it was not left to sit out to dry. This step was eliminated because it exposed the sample to possible environmental contaminants, as was seen by the discovery of starch grains in the controls left out during the trial two analysis.

Two subsamples of each sample were mounted on slides during trials two and three; this was also done during the first two rounds of analysis of the Cis-Baikal samples. The microparticle counts of each of the paired slides were found to be similar. Therefore, it was determined that, with proper vortexing to homogenize the sample within a mounting medium immediately before transfer of a subsample to the slide, each such subsample provides a fairly accurate representation of the sample as a whole. Using the large coverslips drastically increased the amount of time and sample analyzed per subsample, requiring up to 8 hours to scan each subsample under appropriate levels of magnification. Due to time constraints and the limited additional information provided by mounting and scanning the second subsamples, only one subsample was taken from each of the subsequent Cis-Baikal samples unless the slide mount was of poor quality or the detection of little to no calculus material during scanning suggested that any microparticles in the sample had settled before the subsample was drawn and mounted. The remainder of each dissolved and aggregated sample was placed in a refrigerator to slow potential starch degradation and mold growth in the event that an additional subsample had to be drawn, mounted, and scanned for these reasons.

Results were quite poor for trial one. Large chunks of calculus material were observed but few to no plant particles were observed (Table 3.6). Trials two and three yielded much better results than the earlier trials on modern calculus, with the acid proving much more effective at dissolving these older samples and releasing microparticles trapped within them. Both methods yielded microparticles, including starch grains, diatoms and phytoliths (Table 3.6; Figure 3.3),

but the method used in trial three was determined to be the optimal method for plant particle recovery, as high yields of microparticles suggested that the NaHMP was effective in helping to loosen or dislodge particles from the mineral component before demineralization.

Table 3.6 Results of Trials with University of Saskatchewan Osteology Teaching Specimens

Trial	Results
1	Sample 1 and 2: No starch grains, 1-2 possible phytoliths
2	Sample 3: 25+ starch grains and 1-2 possible phytoliths Sample 4: 20+ starch grains, a diatom and 2-4 phytoliths
3	Sample 5: 5+ starch grains and a diatom Sample 6: 30+ starch grains and 1-2 phytoliths

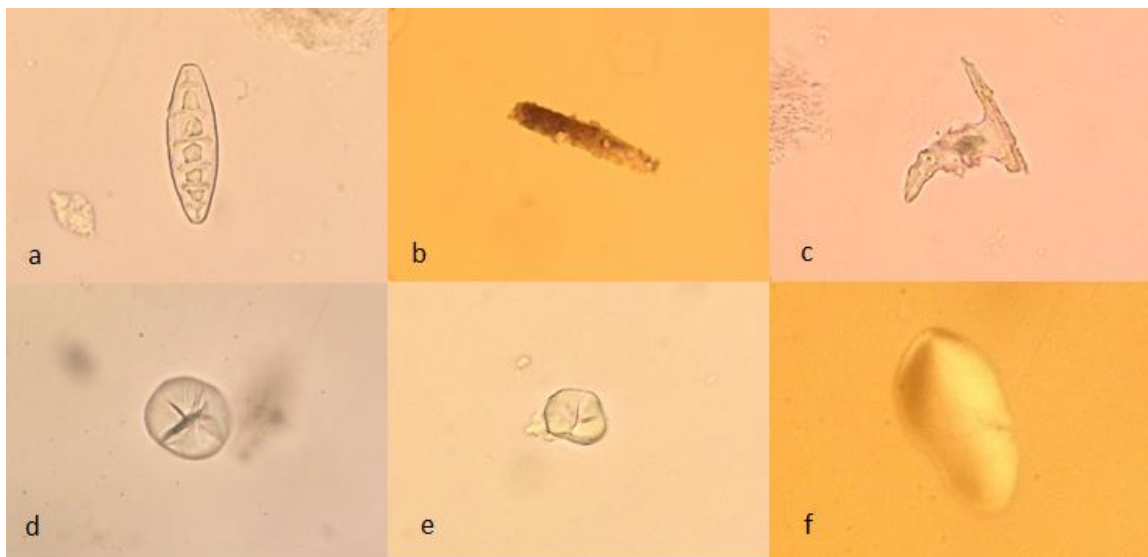


Figure 3.3 Microscopic plant particles extracted from within calculus remains during trials two and three, photos taken at 600X magnification: (a) Diatom recovered during trial two, sample four; (b) Phytolith recovered during trial two, sample four; (c) Phytolith recovered during trial two, sample four; (d) Starch grain recovered during trial two, sample three; (e) Starch grain recovered during trial three, sample six; (f) Starch grain recovered from trial three, sample six.

A single *Lycopodium* tablet was added to the process during the HCl stage. This was a last minute addition to the process, based on Wesolowski et al.'s procedure (2010). All trial calculus samples had been processed by that point so it was tested in blank controls. A

*Lycopodium* tablet contains a known number of *Lycopodium* spores bound together with dissolvable materials. Addition of these tablets makes it possible to determine the total number of plant microparticles within a sample, as the original number of *Lycopodium* spores per tablet versus the number detected during scanning of a subsample can be used to estimate the total number of microparticles in the original sample (see Boyadjian et al. 2007; Wesolowski et al. 2010). If the number of *Lycopodium* spores and plant particles within a subsample are counted, and the number of *Lycopodium* spores added to the whole sample is known, then simple algebra will provide an estimated number of plant particles in the original sample as a whole. University of Lund *Lycopodium* tablet Batch #483216 was used in this trial; its tablets each contain 18,583 *Lycopodium* spores. Their addition appeared workable at this stage, but was discontinued during the processing of the Cis-Baikal samples for reasons outlined below.

Another reason for the addition of the *Lycopodium* spore tablet is that it provides a way to test the effectiveness of the centrifuge and ensure that few to no actual particles from the sample were being pipetted off with the supernatant. Only small numbers (less than 50) of *Lycopodium* spores were found in subsamples of supernatant, suggesting that the methodology was quite effective; however, those small numbers also showed that the centrifuging protocol in use did not ensure that 100 percent of microparticles in each sample were driven out of the supernatant before pipetting. The centrifuge time was therefore increased from five to seven minutes and the speed was increased from 3,500 to 6,000 rpm to create a more compact plug of sample in the bottom of the microcentrifuge tubes. Subsequent examination of pipetted supernatant from samples spiked with *Lycopodium* tablets showed that the recovery rate was still not 100 percent, but the presence of fewer spores in the supernatant was helpful in reducing the number of plant microparticles lost in this fashion.

For the sample processing trials and the first processing round of Cis-Baikal samples, a number of contamination controls were in place in the lab at the University of Saskatchewan. The lab itself was not ideal for preventing contamination. It is located in the Archaeology and Anthropology Building of the University of Saskatchewan, originally built in 1937 (University of Saskatchewan Archives 2015). The age of the building meant that the lab was a repurposed room that had never been established as a contaminant-free environment. An attempt was made to find a more modern positive-pressure or starch-free facility, but no options became available. The lab in the Archaeology and Anthropology Building contained a fume hood, and, while not

positive pressure, it provided a workspace that could be isolated from the rest of the lab. No food or drink was allowed in the lab and its work surfaces were wiped down with a bleach solution everyday using a synthetic cloth. Samples were only opened within the fumehood and were only opened long enough to pipette out or add solution.

All containers and microscope slides were soaked in undiluted bleach for a minimum of three hours then rinsed with RO water. Aluminum foil weigh boats were also soaked in pure bleach but only for half an hour because they began to drastically corrode after that point. All equipment and solutions were stored either within the fumehood or within a specifically designated cabinet. Sterile powder-free gloves were used when calculus samples were being handled directly, while non-sterile powder-free gloves were used during indirect handling (e.g., pipetting). Powder-free gloves were important because the powder in powdered gloves consists of corn starch (Torrence 2006b).

Lastly, in order to determine that starch grains found in the samples were ancient in origin and not simply a contaminant from the laboratory environment, one blank control was included with every round of sampling; these blanks were comprised of a microcentrifuge tube filled with the various processing solutions but containing no sample material. Every step of the established methodology was done in an identical manner to the blanks as to the actual samples. When analyzed microscopically, an absence of plant particles within the blank control samples was taken to indicate that measures used to control for contamination during processing of the associated batch of samples were effective (Zarrillo 2012; Zarrillo and Kooyman 2006:485).

#### *3.2.4 Analysis of Cis-Baikal Material*

Cis-Baikal samples were analyzed generally in groups of two or three and each group was referred to as a “round”. Only samples associated with the same cemetery populations were analyzed together in a round. As mentioned, one blank control was run with each round of samples.

Based on the results of the trials, the methods of trial three were used, including the addition of a *Lycopodium* tablet, to begin the first round of analysis on materials from the Cis-Baikal. The results of round one were not ideal (Chapter 5, Section 5.1; Appendix D, Table D-3). Seven starch grains were found within the blank control, which was very problematic. The lab was subsequently shut down so that controls could be run to investigate where the contamination was coming from. All solutions used in analysis were sampled, swabs were taken of surfaces,

and particle traps were left out in an attempt to determine where contaminants were originating. From these tests, it was found that contamination was occurring late in the process, most likely from coverslips and slides after they were rinsed, as they would behave essentially as a particle trap, gathering adherent airborne particles when wet. To overcome this problem all slides and coverslips were autoclaved before use. While that resolved the problem with plant microparticle contamination, the autoclave bags left a residue likely associated with the fibers from which they were made. These fibers were generally easy to pick out in the samples and were disregarded from analysis.

A plastic barrier was also added around the fumehood to reduce the potential for airborne environmental contaminants (Figure 3.4). This allowed for better isolation of the work area. All handling of the samples was done within the fume hood. An air purifier with a filter capable of trapping particles larger than five  $\mu\text{m}$  was placed within the barrier and ran periodically before starting rounds of sample processing.



Figure 3.4 Isolated work area around fumehood.

Lastly, the optimal plant extraction method was altered to limit the exposure of the sample to the modern environment as much as possible. In the end, a slightly modified version of trial two was used and it was decided to no longer include *Lycopodium* tablets. The final methodology consisted of the following steps:

1. Weigh and crush the sample, except for very small samples or those already in powder form.
2. Add 1.5 mL of HCl (10 percent) until the sample is dissolved (generally between 2 to 24 hours).
3. Rinse with RO water twice, centrifuging at 6,000 rpm for seven minutes to remove supernatant each time.
4. Add 50 percent glycerol and RO water to roughly 0.20 mL and vortex to homogenize.
5. Pipette a sample of roughly 0.10 mL onto a slide, mount, and seal.

As mentioned, the conditions of the Irkutsk State University lab and the University of Saskatchewan lab made it extremely difficult to maintain a contaminant-free environment. Working in a laboratory with a similar history of repurposing and lack of positive pressure fume hood, Zarrillo (2012) found that it was almost impossible to completely eliminate contamination. Therefore all controls, including blanks ran during processing at the University of Saskatchewan, particle traps from the University of Saskatchewan and Irkutsk State University labs, and element/container swabs from the Irkutsk State University lab, were monitored to identify contaminants at all stages of sampling and processing. All calculus samples with associated controls containing contaminant particles were regarded very cautiously. The controls were used to create a reference collection of possible contaminants that each sample may have been exposed to (Appendix D). All particles found in samples were compared to the contaminant reference collection and, if any were found to match a contaminant, they were eliminated from analysis.

Mounted subsamples were scanned with an Olympus CX41 light microscope, with magnifications from 40 to 600X. Not only were particles examined within the trial and Cis-Baikal samples, but comparative modern phytoliths and starch grains were examined as well (see Section 3.2.5). Based on size and observability of particles found in the trial rounds and mounts



bearing modern phytoliths and starch grains, it was decided to scan mounted samples at 100 and 200X magnification and look at details at 400 and 600X magnification. Most photographs were taken at 600X magnification. Polarizing light filters were used to create cross polarized light for the identification of starch grains (see Section 3.2.5).

### *3.2.5 Phytolith and Starch Identification*

The researcher had no prior experience with identifying microscopic plant particles; therefore it was important look at modern comparatives to become comfortable identifying particles before analysis began.

Examples of a broad range of phytoliths were observed in mounted soil samples provided by Lauren Stead at the University of Saskatchewan and in dry-ashed plant samples provided by Dr. Brian Kooyman at the University of Calgary, as well as by looking at multiple online databases (Pearsall 2011; Piperno and Kealhofer 1998; Phytcore 2014). These sources were used to create a list of diagnostic characteristics of phytoliths. Key characteristics observed included: light brown to opaque or translucent colour, often with a pinkish hue; three-dimensional structures based on cell shape and defined borders (often seen as a double border through a microscope); central void often present in body; and no birefringence (see Chapter 2, Figure 2.2). Every characteristic listed did not need to be present for a particle to be recorded as a phytolith. It was particularly problematic if a particle could not be rotated within the fluid mounting medium using gentle pressure on the cover slip. In these instances, only one plane of the particle could be seen; it often could not be confidently identified as a phytolith and therefore was listed only as a “possible” phytolith. When a particle was identified as a phytolith or a possible phytolith, its features were carefully described (size, colour, three-dimensional shape, etc.) and photographed.

Modern comparative samples were created to gain experience in identifying starch. This was done by crushing food samples such as corn, potato, yam, and pine nuts, and mounting them on slides. The McCrone Atlas of Microscopic Particles (The McCrone Group 2005-2012) was also consulted extensively. While stains such as iodine can be used to identify starch (Gott et al. 2006), these could not be used in this study because the mounting medium did not allow the stain to travel through the slide; the dark colour of the stain also permanently obscures some of the other diagnostic features of starch grains. Therefore, identification was based purely on observation of unstained particles.

Starch grains are composed primarily of glucose, formed into two types of chains: amylopectin and amylose (Gott et al. 2006). Formation of the starch grain begins at the hilum, or central growth point, and is laid down in layers successively creating rings called lamellae, similar to a tree (Gott et al. 2006), although the hilum is not always in the center of the grain. Lamellae are composed of alternating layers of crystalline and amorphous material, with varied ratios of amylopectin and amylose depending on genetic and environmental factors. They give the starch grain a semi-crystalline structure and birefringence with a characteristic extinction cross under cross polarized light (Gott et al. 2006; Haslam 2004). Starch grains can be quite variable, but these are general properties that clearly and consistently characterize most of them (Figure 3.5). Other characteristics common for starch grains include fissures and pores on their surfaces.

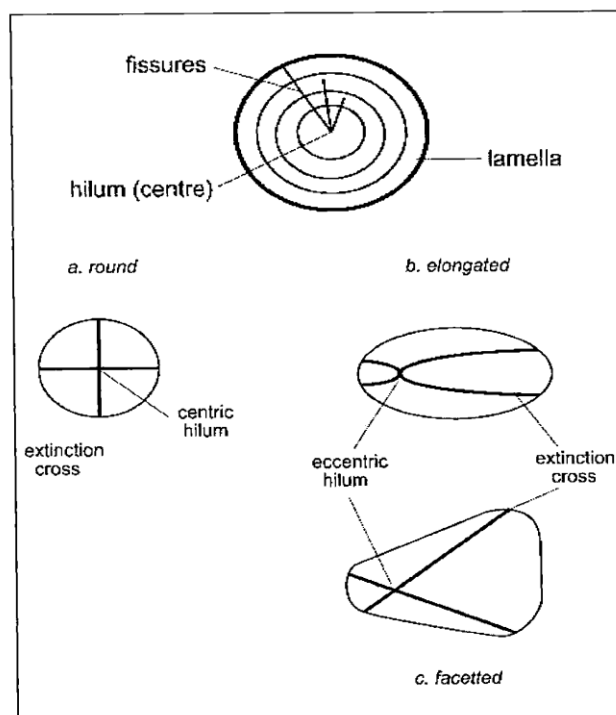


Figure 3.5 Key characteristics of a starch grain (Gott et al. 2006:Figure 2.9, illustration by Fiona Roberts).

The most important indicator of starch grains is that they exhibit strong birefringence: they light up bright white and exhibit an extinction cross when viewed under cross polarized light. This pattern occurs due to the semi-crystalline arrangement of starch molecules, which

causes the polarized light to pass through the grain at different velocities (Gott et al. 2006). The arms of the extinction cross meet at the hilum and the cross will rotate with the polarizing lens (Gott et al. 2006; Figure 3.5). All unmodified starch grains will exhibit this property, although the strength of the cross may vary from strong and sharp to weak and blurred (Torrence 2006a). Other particles such as faecal spherulites, crystalline spherulites, some spores, cellulose, some partially fractured exines of pollen granules and coccoliths can exhibit something similar to an extinction cross, but they can usually be distinguished from starch grains by other physical characteristics, such as lack of a rotating cross, the presence of a rainbow effect under polarized light and the overall shape of the particle (Torrence 2006a). A list of features, outlined in Gott et al. 2006, Torrence 2006a and Torrence 2006b, was used to aid in identification and description of starch grains observed in the Cis-Baikal samples (Table 3.7).

Table 3.7 Descriptions of Starch Grains

Feature	Observation
Hilum	Open or closed; central or acentric
Lamellae	Present or absent
Fissures	Straight, Y-shaped, stellate, etc.
Size	Measured in microns (generally between 1 to 100 $\mu\text{m}$ )
Shape	Round, oval, disc-shaped, lenticular, elongated, kidney-shaped, polyhedral, etc.
Birefringence	Sharp extinction cross, faint extinction cross, wide-armed extinction cross, etc.
Damage	Cracking, broken/torn, disturbance to birefringence, gelatinization, etc.
Other	Indentations, pores, simple or complex grain, etc.

Based on Gott et al. 2006, Torrence 2006a, and Torrence 2006b

Starch grains and phytoliths were the focus of the microscopic analysis section of this thesis, but other particles found within the calculus were also noted and photographed. This included particles such as diatoms, charcoal, spores, and fibers, which have all been previously noted within samples from other calculus research projects (e.g., Dudgeon and Tromp 2012; Blatt et al. 2010; Charlier et al. 2010; Boyadjian et al. 2007; Mickleburgh and Pagan-Jimenez

2012; Wesolowski et al. 2010). Aid in the diagnosis of these particles was provided by Dr. Elizabeth Robertson and Dr. Glenn Stuart, both of the University of Saskatchewan.

### **3.3 Chapter Summary**

To summarize, dental calculus in Cis-Baikal individuals was studied in two distinct ways during this research project: by the macroscopic observation of dental calculus severity within oral quadrants and by microscopic analysis of microparticles that would have become embedded within calculus during the life of the individual. Dental calculus severity was determined by converting common dental surface scores into indices for four quadrants within the mouth, based on Greene et al. (2005). These indices were analyzed statistically to determine similarities and differences within and among four cemetery populations in the Cis-Baikal to address research questions about the utility of dental calculus as an indicator for dietary and mouth use patterns in the Cis-Baikal. For the microscopic analysis, individual calculus samples were dissociated in HCl solutions and all potential contamination was closely monitored. Subsamples were observed with an optical light microscope to recover microparticles from the matrix to determine if this is a feasible strategy for dietary and mouth use reconstruction. Results of the first approach are reported in Chapter 4 while results of the second approach are reported in Chapter 5.

## **Chapter 4**

### **Results and Interpretation of Macroscopic Analysis of Dental Calculus Severity**

The analysis of dental calculus severity can provide important information about aspects of past activity, as outlined in Chapter 2. Calculus formation is known to be influenced by protein and carbohydrate intake and comparing severity rates can therefore provide information on the proportion of each in the diet, reliant on diet being the main factor in calculus formation. Other factors are also known to affect calculus formation, however, such as genetics, environment, and non-dietary mouth use. The advantage for this project is that stable isotope analysis in the Cis-Baikal, discussed in Chapter 1, is well established and indicates that Cis-Baikal individuals from both the EN and LN-EBA had a high protein/low carbohydrate diet, reliant mainly on local fish and terrestrial mammals. Severity differences and trends are compared with the stable isotope results to determine if dental calculus is a good proxy for diet in the Cis-Baikal, or whether some other factor is exhibiting a greater effect. Intra- and intersite analyses were conducted using nonparametric testing, as outlined in Chapter 3.

#### **4.1 Exploring Possible Post Excavation Biases**

There were two main sources of potential bias present within the data: small sample sizes and postmortem removal of calculus. Small samples sizes are known to increase the chances of Type II errors, which involve failing to detect a significant difference during statistical testing when there is a significant difference in reality (Pallant 2010). This is a problem in many archaeological cases because it is often not possible to increase sample sizes; there is a limited quantity of recoverable remains at a site. Here, Ust'-Ida I was not only a much smaller cemetery population than Lokomotiv and Shamanka II, but it only provided three females eligible for inclusion in analyses. Another hindrance to analysis was the much poorer preservation of Khuzhir-Nuge XIV individuals due to high fragmentation rates of skeletal materials. Appendix A, Table A-1 demonstrates that the number of teeth that were present but completely unobservable was much higher for Khuzhir-Nuge XIV than for the other cemeteries. Many teeth were highly fragmented and charred in some cases. This led to poor representation and small

sample sizes, which limited the amount of information that could be gained from this cemetery. This potential sample size bias was not ignored here, and is noted where appropriate below. Visual analysis of boxplots and discussion of basic trends was utilized along with statistical analysis to aid in interpretation in light of limited sample sizes.

There was about a 20-year gap between when Lokomotiv (EN) and Ust'-Ida I (LN-EBA) were excavated and when Shamanka II (EN) and Khuzhir-Nuge XIV (LN-EBA) were excavated. The utility of dental calculus was not fully understood when earlier excavations were taking place and Lieverse et al. (2007a) noted that post-excavation removal of calculus had likely taken place as a result of several factors, including over-exuberant cleaning of teeth, curation damage, and removal of deposits to allow unobstructed measurements and/or photographs of teeth for the former two sites.

It was necessary to look at the distributions from Lokomotiv and Ust'-Ida I to attempt to determine whether the data told us anything meaningful about aspects of the individuals' lives or were now completely biased by post-excavation removal, whether intentional or not. This was determined by looking at the distributions of the data to see if they followed overarching trends established by previous research on dental calculus formation. For example, Chapter 2 explained that salivary glands are located in the anterior mandibular area of the mouth and around the maxillary first molar (Mandel 1990; Clerehugh et al. 2009). Also, intuitively, saliva pools in the mandible. Minerals from saliva are a key component of dental calculus (Mandel 1990) and therefore areas with increased saliva accumulation should form more calculus. Thus, the LA and UP quadrants should accumulate the greatest calculus deposits (White 1997; Parfitt 1959; Jin and Yip 2002). The LP quadrant should also accumulate calculus deposits readily due to saliva pooling. The UA quadrant is not located next to any salivary gland and it not subject to saliva pooling; therefore, it should have the smallest deposits when compared to the other quadrants. Another trend that should be observed within the data set is increasing dental calculus accumulation with age. This is due to the age-progressive nature of dental calculus formation, which is well established in dental literature if regular dental cleaning is not performed (White 1997; Anerud 1991). There should therefore be an increase in calculus accumulation from juveniles to young adults and from young adults to old adults.

For Lokomotiv, distributions for all individuals, with no age or sex separations, within each quadrant generally corresponded well to these established trends; the LA quadrant

contained the largest indices overall and the UA quadrant contained the lowest (Figure 4.1). In contrast, when looking at only juveniles, this trend was not evident, although calculus deposits were all very small to absent (Figure 4.2). Postmortem cleaning may have biased the juvenile sample, especially since the sample size was quite small ( $n \leq 8$ ). There was a drastic spike in calculus formation from juveniles to adults (Figure 4.2); consequently, the age progressive nature of calculus formation was upheld. On the other hand, the typical increase in calculus from young adults to old adults was negligible within this data set overall. This is attributed to the removal of deposits due to extensive wear to the teeth rather than post-excavation removal. Although good evidence existed that some deposits were removed from the teeth post-excavation, it seemed that the overall trends in the data were likely intact and could still be meaningful to analysis.

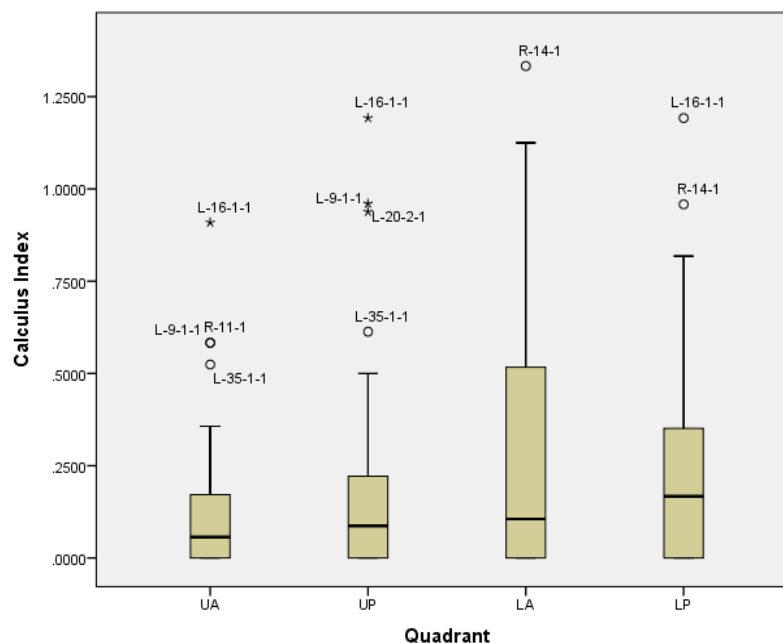


Figure 4.1 Distribution of dental calculus indices for all individuals from Lokomotiv. Note: Open circles represent “out values” while stars represent “far out values” that are more likely to influence data outcomes. It is realized that these are potentially problematic but there was no justification for their removal. This applies to all other box plots presented hereafter.

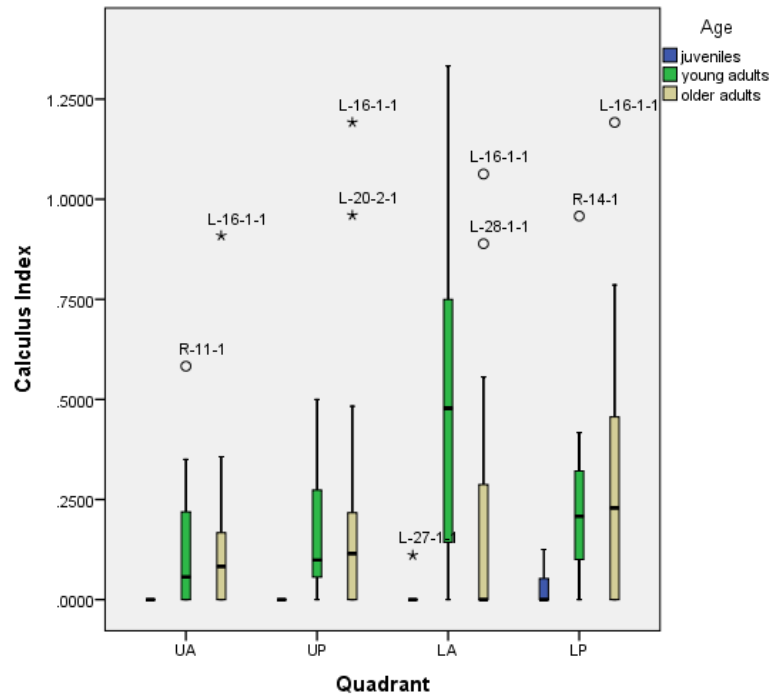


Figure 4.2 Distribution of dental calculus indices from Lokomotiv, separated by age only.

The distribution of data, with no distinctions made in the data between ages or sexes, from Ust'-Ida I individuals also corresponded quite well with typical locations of calculus formation (Figure 4.3). Deposits located in the LA quadrant had the highest indices overall and the UA quadrants had quite low accumulation comparatively (Figure 4.3). The indices from the UP quadrant showed some possible problems with the data, however. Most individuals had no calculus present (median=0.000) and a large number of individuals with deposits were considered outliers. Based on the distributions of data from the other quadrants, greater severity in the UP quadrant was expected, since it should have been one of the first areas to form calculus. Since this was not the case, it may indicate some postmortem removal of deposits. Figure 4.4 provides some further support for this. The increase in deposits was seen from juveniles to adults, and for the UA and LP quadrants, from young to old adults, but for the UP quadrant the calculus indices for old adults dropped back to zero, except for one individual. Therefore, again, good evidence existed that calculus had been removed from tooth surfaces, but the overall trends remained intact, except for potentially the UP quadrant. Thus, while the data were regarded cautiously, they still provided useful information.



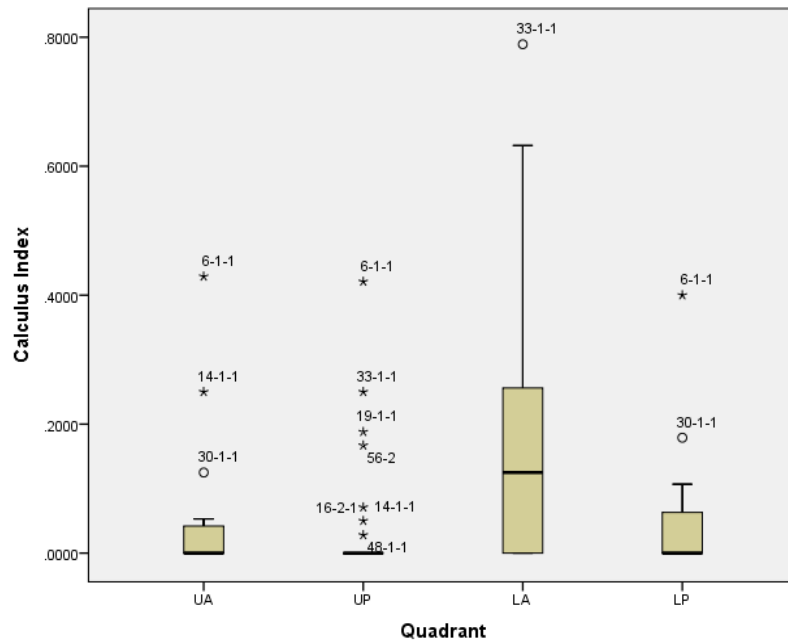


Figure 4.3 Distribution of dental calculus indices from all individuals from Ust'-Ida I.

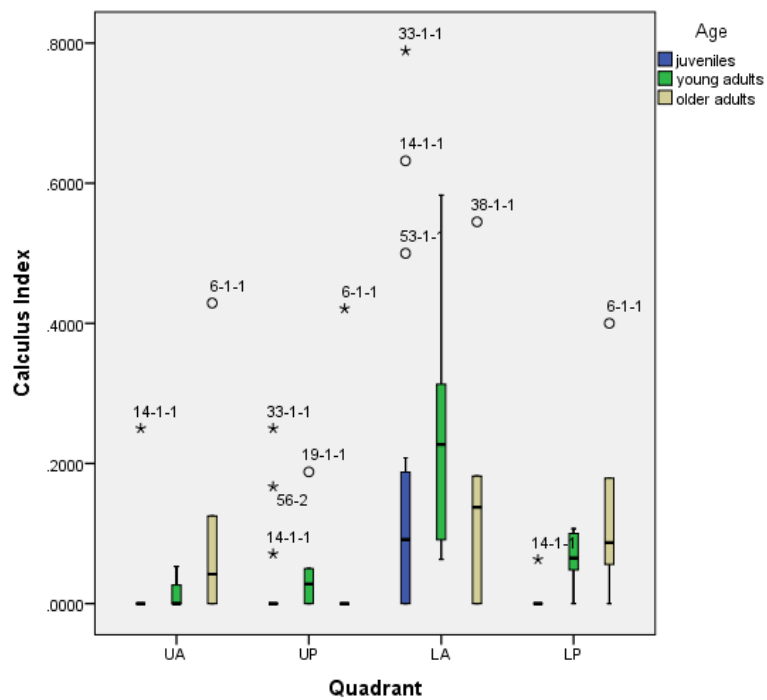


Figure 4.4 Distribution of dental calculus indices from Ust'-Ida I, separated by age only.

## 4.2 Results of Intrasite Analyses of Calculus Severity

Intrasite statistical analysis was performed only on adults from each of the cemetery populations. As discussed in Chapter 3, Section 3.1.3, it was decided to separate juveniles from adults in the analysis due to developmental differences likely affecting variance and the relationship between dependent and independent variables. Intrasite statistical analysis of age consequently consisted of the comparison of only young and old adults. The intrasite statistical analysis of sex consisted only of the comparison of adult males and adult females; sample sizes were too small and/or sex was not determinable within the juvenile group to compare males and females. Observations regarding juveniles were based on visual analysis of boxplots only.

Excluding juveniles meant that all comparisons were between only two sample populations; as a result, Mann-Whitney U tests were used exclusively for the intrasite analysis. Differences were considered significant when  $p < 0.05$ . SPSS computes an asymptotic (Asymp.) p-value when sample sizes (n) are larger than 30, which corrects for ties in the ranked data (Pallant 2010:228-229). If the sample size is below, or close to 30, though SPSS will also calculate an exact p-value, which will compute the test based on the exact distribution of the data regardless of ties, to maintain accurate p-values despite problems inherent with small sample sizes (Mehta and Patel 1989:1). For the Cis-Baikal data, if an exact and an Asymp. p-value were both calculated due to the sample size, the exact p-value was used preferentially. Tests related to Shamanka II individuals had sufficiently large sample sizes that an exact p-value was not generated. A table of the results is provided within Table 4.1.

### 4.2.1 *Lokomotiv (EN)*

No difference between adult males and females was detected within this cemetery for any quadrant ( $p=0.941$ ,  $0.448$ ,  $0.842$ , and  $0.200$  for UA, UP, LA, and LP respectively). Though not significantly different, females showed a higher median calculus index for the UP and LA quadrants (Table 4.1). These were the only two cases within all of the cemeteries where females exhibited higher calculus indices overall than males.

No significant difference was seen between young and old adults for the UA, UP, and LP quadrants ( $p=0.732$ ,  $0.857$ , and  $0.952$  respectively). However, a significant difference ( $p=0.006$ ) was seen between young and old adults for the LA quadrant. Young adults had significantly larger calculus severity (median= $0.583$ ) than older adults (median= $0.034$ ; Table 4.1).

Table 4.1 Intrasite Analyses to Determine Differences in Dental Calculus Severity

Category	Cemetery	Quadrant	n	Z score	Medians	p-value (exact)
Sex (males/females)	LOK	UA	27 (17/10)	-0.102	0.136/0.115	0.941
		UP	30 (20/10)	-0.797	0.099/0.152	0.448
		LA	29 (18/11)	-0.205	0.125/0.250	0.842
		LP	31 (17/14)	-1.302	0.259/0.089	0.200
	SHA	UA	51 (39/12)	-1.979	0.375/0.192	0.048**
		UP	53 (40/13)	-2.088	0.345/0.176	0.037**
		LA	51 (38/13)	-1.016	0.711/0.333	0.309**
		LP	53 (39/14)	-2.099	0.429/0.354	0.036**
	UID	UA	9 (6/3)	-0.283	0.021/0.000	0.905
		UP	11 (8/3)	-1.419	0.014/0.000	0.279
		LA	12 (9/3)	-0.649	0.150/0.150	0.600
		LP	11 (8/3)	-1.023	0.084/0.000	0.376
	K14	UA	8 (8/0)	*	0.061/*	*
		UP	8 (7/1)	*	0.273/0.176	*
		LA	5 (5/0)	*	0.308/*	*
		LP	11 (10/1)	*	0.556/0.125	*
Sex (males/probable males)	SHA	UA	39 (36/3)	-0.554	0.375/0.125	0.599
		UP	40 (37/3)	-1.670	0.343/0.613	0.104
		LA	40 (38/2)	*	0.711/1.239	*
		LP	41 (39/2)	*	0.429/0.973	*
	K14	UA	8 (5/3)	-0.153	0.063/0.059	1.000
		UP	9 (7/2)	*	0.273/0.234	*
		LA	7 (5/2)	*	0.308/0.273	*
		LP	10 (7/3)	-0.342	0.476/0.636	0.833
Sex (females/probable females)	SHA	UA	13 (12/1)	*	0.192/0.250	*
		UP	15 (13/2)	*	0.176/0.180	*
		LA	14 (13/1)	*	0.333/0.500	*
		LP	15 (14/1)	*	0.354/0.000	*

Category	Cemetery	Quadrant	n	Z score	Medians	p-value (exact)
Age (young adults/ old adults)	K14	UA	0	*	*	*
		UP	2 (1/1)	*	0.176/0.333	*
		LA	1 (0/1)	*	*/0.000	*
		LP	2 (1/1)	*	0.125/0.000	*
	LOK	UA	28 (12/16)	-0.357	0.057/0.140	0.732
		UP	31 (12/19)	-0.204	0.099/0.154	0.857
		LA	29 (11/18)	-2.738	0.583/0.034	0.006
		LP	31 (12/19)	-0.061	0.208/0.208	0.952
	SHA	UA	52 (35/17)	-0.264	0.261/0.333	0.792**
		UP	55 (38/17)	-1.211	0.270/0.456	0.226**
		LA	55 (35/20)	-1.505	0.800/0.511	0.132**
		LP	57 (38/19)	-1.854	0.361/0.611	0.064**
	UID	UA	9 (4/5)	-1.073	0.000/0.042	0.413
		UP	11 (5/6)	-1.057	0.028/0.000	0.429
		LA	12 (6/6)	-0.884	0.227/0.138	0.394
		LP	11 (6/5)	-0.823	0.065/0.087	0.429
	K14	UA	10 (5/5)	-0.216	0.059/0.063	0.841
		UP	13 (6/7)	-0.429	0.302/0.273	0.731
		LA	10 (6/4)	-0.863	0.354/0.063	0.476
		LP	15 (7/8)	-0.348	0.476/0.213	0.779

\*No data;\*\*Larger sample size therefore asymptotic p-value calculated; n= sample size; Significance when  $p < 0.05$  (highlighted in gray); LOK=Lokomotiv; SHA= Shamanka II; UID=Ust'-Ida I; K14=Khuzhir-Nuge XIV

Juveniles displayed very low severity overall. Calculus indices were constant, with a median at 0.000 for the UA, UP, and LA, except for one outlier in the LA quadrant. The LP quadrant had slightly greater variation but with a median still at 0.000. These low indices may have been affected by postmortem calculus removal.

#### *4.2.2 Shamanka II (EN)*

Shamanka II contained a number of probable males and females that could not be confidently assigned to either group. As noted in Chapter 3, in order to be included they needed to display no statistically significant differences from confidently identified males or females. There were not enough probable females ( $n < 3$ ) to run statistical tests to see if there was any difference between females and probable females. Therefore, probable females were not included in the analysis. Probable males were included for two quadrants, UA and UP ( $n = 3$ ; Table 4.1). In both cases, no significant difference was found between probable males and confirmed males ( $p = 0.599$  and  $0.104$  respectively; Table 4.1) and these samples were pooled for analyses. Sample sizes for probable males were not large enough for the other quadrants; they were discarded for this analysis.

Adult males were found to have significantly higher calculus indices than females in three out of four quadrants ( $p = 0.048$ ,  $0.037$ , and  $0.036$  for UA, UP, and LP respectively; Table 4.1). While males exhibited greater calculus severity overall for the LA quadrant, this difference was not statistically significant ( $p = 0.309$ ).

No statistical significance was found when comparing young and old adults, when  $p < 0.05$  was maintained ( $p = 0.792$ ,  $0.226$ ,  $0.132$ , and  $0.064$  for UA, UP, LA and LP respectively). Juveniles consistently had the lowest indices, with much less variance than the adult groups. Despite non-significance, old adults had larger calculus deposits for all but the LA quadrant.

#### *4.2.3 Ust'-Ida I (LN-EBA)*

For the most part, calculus deposits were very small or absent for Ust'-Ida I, with calculus indices generally around 0.000. Variation in the data was quite less than was seen for Shamanka II and Lokomotiv. Therefore, any moderate deposits ended up being outliers within the dataset (Figure 4.4). The outliers were likely more of a product of the small sample size for this cemetery than errors in scoring.

While not significantly different, males exhibited greater calculus severity than females for every quadrant ( $p=0.905$ ,  $0.279$ ,  $0.600$ , and  $0.376$  for UA, UP, LA and LP respectively; Table 4.1). Samples sizes for females were small however ( $n=3$ ; Table 4.1). Similarly, no significance was detected during age comparisons, although younger adults had slightly larger indices for the UP and LA quadrants ( $p=0.429$  and  $0.394$  respectively) and older adults had slightly larger indices for the UA and LP quadrants ( $p=0.413$  and  $0.429$  respectively).

#### *4.2.4 Khuzhir-Nuge XIV (LN-EBA)*

Comparisons between males and females were not conducted due to the female sample population being too small (Table 4.1). Comparisons were made between probable males and confidently identified males for the UA and LP quadrants, with no significant difference being detected ( $p=1.000$  and  $0.833$  for UA and LP respectively). Based on this, probable males were combined with the male group for the intersite analysis for the UA and LP quadrants. Samples sizes were too small to compare the UP and LA quadrants.

Young adults displayed greater calculus severity than older adults for every quadrant, though none of these differences was statistically significant ( $p=0.841$ ,  $0.731$ ,  $0.476$ , and  $0.779$  for UA, UP, LA, and LP respectively). Juveniles had consistently lower indices.

#### *4.2.5 Summary*

Overall, significant differences between males and females were only seen within the cemetery population from Shamanka II. The other cemeteries had smaller sample sizes, which may have had an effect on the ability to detect differences by the chosen statistical methods. Males generally exhibited greater calculus severity than females, regardless of significance, with the possible exception of Lokomotiv.

Except for the LA quadrant of Lokomotiv individuals, no difference was seen statistically between young and old adults. As often as not, young adults exhibited higher calculus indices than old adults, including at Lokomotiv for the LA quadrant, where individuals had significantly more calculus as young adults than as old adults ( $p=0.006$ ).

#### *4.2.6 Interpretation and Significance*

From a dietary standpoint, the lack of differences between sexes suggests that males and females from Lokomotiv, Ust'-Ida I, and Khuzhir-Nuge XIV were not eating significantly different proportions of meat to carbohydrates, provided that diet is the primary driver in

calculus formation. It may reflect similar access to resources and thus little to no social differentiation between sexes or it may simply reflect that all individuals regardless of sex were consuming large quantities of fish and terrestrial game.

These results correspond well with previously obtained information on the LN-EBA ISG populations (Ust'-Ida I and Khuzhir-Nuge XIV), where less social differentiation was noted and everyone was consuming predominately terrestrial game and fish (Lieverse 2010; Waters-Rist 2011; Weber and Bettinger 2010; Katzenberg and Weber 1999). On the other hand, despite the fact that EN individuals should have been eating predominately similar proportions of protein, significant differences were noted between adult males and females at Shamanka II and not Lokomotiv. Greater social differentiation was noted in EN Kitoi populations through previous bioarchaeological investigations (Lieverse 2010; Weber and Bettinger 2010) and may have extended to differences in the quantities or types of food consumed or other mouth use practices that can increase or decrease calculus severity. This dichotomy between these EN cemeteries has been previously documented in studies of activity patterns (Lieverse et al. 2013; Lieverse et al. 2015). Lokomotiv's failure to show this dichotomy in its results may be the consequence of a smaller sample size, increasing the chances of Type II errors, or it may suggest one, or a combination, of three things.

First, it may suggest that diet was not the primary factor affecting calculus accumulation within the population, but instead environmental factors or individual variation may have played roles. Though they likely interacted to some extent (Katzenberg et al. 2010), individuals from Lokomotiv remained predominately within the Angara River Valley microregion, while those from Shamanka II inhabited the South Baikal microregion. They were likely exposed to different environments with variations in factors like water mineral content, which are known to affect calculus accumulations (Gaare et al. 1989; Lieverse 1999; Damen and ten Cate 1989). Kitoi males were thought to have engaged in more logistic foraging while females remained more sedentary around habitation centers close to fisheries (Weber 1995; Katzenberg and Weber 1999; Haverkort et al. 2008; Lieverse et al. 2007b). Still, despite variations in male and female time spent at those centers, some aspect of the Lokomotiv home base area, where both males and females interacted, may have had an overriding effect on calculus accumulations, causing severity to be similar, masking any influence of diet or other factors associated with gendered activity. However, for the Shamanka II population, gendered differences in environmental

exposure due to varying mobility may have generated the observed variations in male versus female calculus severity.

Second, it may suggest that, despite social differentiation in some aspects of life, males and females at Lokomotiv were less socially differentiated in contrast to EN Kitoi individuals at Shamanka II, with that extending to diet and/or other factors that may have influenced calculus formation. This possible explanation is interesting because the lack of differences between Lokomotiv males and females had also been noted during activity-related studies on Cis-Baikal individuals. Lieverse et al. (2013) reported increased sexual disparity on lower limb enthesal markers with age for all cemeteries studied, except Lokomotiv. At Lokomotiv, differences were much less pronounced, similar to the results seen here, and it was attributed to increased activity levels utilizing the lower limb in both females and men, while the other cemeteries showed an increase in activity levels in males but much less so in females (Lieverse et al. 2013). It is important to note that these sex differences were only noted by some activity pattern studies, while others noted greater sexual disparity related to other skeletal areas (e.g., Stock et al. 2010; Lieverse et al. 2010; Lieverse et al. 2009).

Third, post-excavation cleaning activities may have eliminated differences. Section 4.1.1 confirmed that while overarching trends still appeared to be present within Lokomotiv individuals, removal of calculus had affected calculus severity indices to at least some extent.

In contrast, the results of the intrasite analysis for Shamanka II support previous research on social differentiation between males and females within the Kitoi culture. The results may suggest that EN Kitoi males received a larger portion of protein compared to other foodstuffs than females or that they were eating specific resources in larger quantities (e.g., terrestrial versus freshwater resources and/or seal) that may have had an effect on the oral environment or calculus formation to a greater extent. Again, logistical foraging may be the key to these differences. Logistic foraging would have forced males out of local home base settings, exposing them to new environments and food sources (Lieverse et al. 2007a). They likely had a different diet in hunting parties than at their home base and this may be reflected in the dental calculus.

On the other hand, any such dietary differences between males and females not been noted in stable isotope analysis. The new environments males were exposed to through logistical food procurement strategies may have had just as much of an effect, if not more, on dental calculus severity. As mentioned in the case of Lokomotiv, factors such as water sources can



influence calculus formation (Lieverse et al. 2007a). Shamanka II females would have been exposed to water from Lake Baikal and its immediate tributary rivers, while males would have moved further away from this system. They may have encountered different riverine and lake systems with differing mineral concentrations.

The results from the analysis of differences between young and older adults within each of the cemetery populations were originally quite surprising, since dental calculus is an age progressive condition, if the deposits are not removed (White 1997; Anerud et al. 1991). These results instead suggest that the calculus was being removed from the teeth as individuals aged; this is presumably due to dental wear for the Cis-Baikal populations. Lieverse et al. (2007a) stated that dental wear was pronounced for all four cemetery populations, with initial dentin exposure on anterior teeth often occurring during adolescence. They noted increasing attrition with advancing age at death for all tooth types, without exception. Waters-Rist et al. (2010) noted the presence of activity-induced dental modifications to the occlusal surface of teeth in 20.3 percent of individuals from five Cis-Baikal cemeteries (Lokomotiv, Shamanka II, Ust'-Ida I, Khuzhir-Nuge XIV, and Kurma XI). It was concluded that occlusal grooves were likely caused by repeated friction from running plant fibers and sinew over the surface (Waters-Rist et al. 2010). An abrasive diet and/or non-dietary mouth use causing attrition over time would potentially wear off the calculus deposits attached to the tooth surface, removing and preventing further accumulation. Discussed earlier was the issue of how to score these severely worn teeth since the tooth surfaces were massively reduced. It was decided that, if postmortem damage was not evident it would be scored regardless of the amount of wear, since calculus can accumulate on the root as well as the crown. These results were the direct effect of the decision to include teeth with extensive dental wear.

#### **4.3 Results of Intersite Analyses of Dental Calculus Severity**

Due to the differences observed between multiple quadrants in males and females from Shamanka II in the intrasite analysis, all adult males and females were analyzed separately for intersite analyses. There was only one significant difference found in one quadrant at one cemetery between young and old adults from intrasite analyses; therefore, due to the general lack of differences, these two groups were combined for intersite analyses. All tests involved comparisons of more than two cemeteries and, as such, Kruskal-Wallis tests were performed. SPSS generates an asymptotic p-value for Kruskal-Wallis testing unless samples sizes are less

than 15 (Mehta and Patel 2011:5). All tests conducted contained sample sizes larger than 15; therefore the asymptotic p-value was recorded from each test on Cis-Baikal materials. When significant differences were noted, stepwise step-down post hoc testing was used to determine where these differences were coming from. Recall that stepwise step-down analysis generates homogenous subset tables. Variables that appear in the same subset are considered to not be significantly different, while variables that do not appear in the same subset are significantly different ( $p < 0.05$ ). A summary of the results of this analysis is provided in Table 4.2.

#### *4.3.1 Juveniles*

Males and females were not differentiated when comparing juveniles due to small sample sizes and the fact that most subadults have not undergone hormonal changes that aid in sex determination on skeletal data (Byers 2011:169). All four cemeteries were compared.

##### *4.3.1.1 Upper Anterior Quadrant*

Based on observations from the boxplot presented in Figure 4.5, juveniles from Shamanka II had the largest calculus deposits, as well much greater variation in indices among individuals within the cemetery. Despite this, statistical analysis revealed no significant difference between individual calculus indices from each of the four cemetery populations ( $p = 0.131$ ; Table 4.2).

##### *4.3.1.2 Upper Posterior Quadrant*

Shamanka II can be seen to have had the highest median value (Figure 4.6a; Table 4.2) though Khuzhir-Nuge XIV had greater variation in indices and had a slightly higher rank during Kruskal-Wallis testing (Figure 4.6b).

Overall, a significant difference was found when the four cemeteries were compared together ( $p = 0.001$ ). The results of post hoc testing are presented in Figure 4.6b. Lokomotiv was ranked with the lowest calculus severity overall. Shamanka II and Khuzhir-Nuge XIV were both significantly different from Lokomotiv and Ust'-Ida I but neither Shamanka II and Khuzhir-Nuge XIV or Lokomotiv and Ust'-Ida I were significantly different from each other ( $p = 0.979$  and  $0.407$  respectively).

Table 4.2 Intersite Analyses to Determine Differences Between Cemetery Groups

Category	Quadrant	n	$\chi^2$	df	Medians	p-value (Asymp.)
Juveniles (LOK/SHA/UID/K14)	UA	35 (4/12/8/11)	5.639	3	0.000/0.050/0.000/0.000	0.131
	UP	55 (8/17/18/12)	15.415	3	0.000/0.083/0.000/0.0565	0.001
	LA	49 (6/13/16/14)	8.743	3	0.000/0.250/0.0915/0.0665	0.033
	LP	47 (7/11/15/14)	13.57	3	0.000/0.143/0.000/0.019	0.004
Males (LOK/SHA/UID/K14)	UA	70 (17/39/6/8)	14.74	3	0.136/0.375/0.0210/0.0610	0.002
	UP	75 (20/40/8/7)	19.383	3	0.099/0.345/0.014/0.273	0.000
	LA	70 (18/38/9/5)	12.746	3	0.125/0.7105/0.150/0.308	0.005
	LP	74 (17/39/8/10)	12.523	3	0.259/0.429/0.0835/0.556	0.006
Females (LOK/SHA/UID)	UA	25 (10/12/3)	4.204	2	0.115/0.192/0.000	0.122
	UP	26 (10/13/3)	6.819	2	0.1515/0.176/0.000	0.033
	LA	27 (11/13/3)	1.987	2	0.250/0.333/0.150	0.370
	LP	31 (14/14/3)	4.038	2	0.0885/0.3535/0.000	0.133

Asymp.=Asymptotic p-value; Significance when  $p < 0.05$  (highlighted in gray); LOK= Lokomotiv, SHA= Shamanka II, UID= Ust'-Ida I, K14= Khuzhir-Nuge XIV

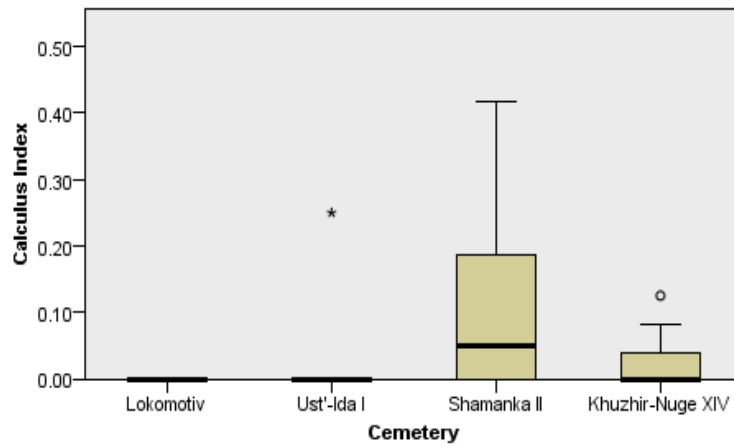
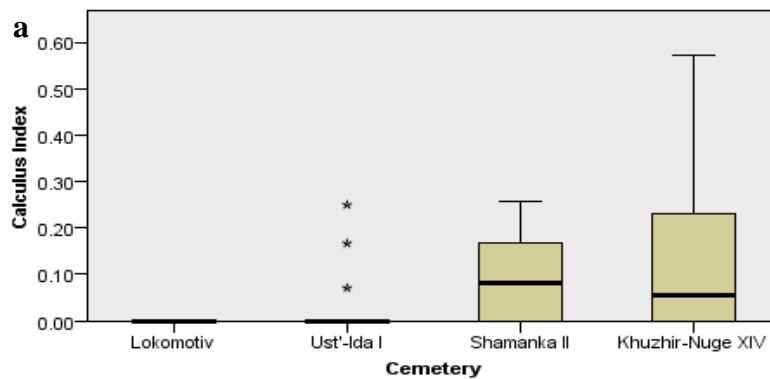


Figure 4.5 Distribution of UA calculus indices from juvenile individuals from Cis-Baikal populations.



<b>b</b>		Subset	
		1	2
Sample <sup>1</sup>	Lokomotiv	17.000	
	Ust'-Ida I	21.667	
	Shamanka II		34.618
	Khuzhir-Nuge XIV		35.458
Test Statistic		1.443	.033
Sig. (2-sided test)		.230	.856
Adjusted Sig. (2-sided test)		.407	.979

Homogeneous subsets are based on asymptotic significances. The significance level is .05.

<sup>1</sup>Each cell shows the sample average rank of Calculus Index.

Figure 4.6 Results from analysis of UP calculus indices from juvenile individuals from all Cis-Baikal cemeteries: (a) Distribution of indices per cemetery; (b) Results of post hoc analysis.

#### 4.3.1.3 Lower Anterior Quadrant

Shamanka II individuals exhibited the highest median index (0.250) and also the highest rank value during Kruskal-Wallis testing (Figure 4.7a, b; Table 4.2). Lokomotiv individuals exhibited the lowest indices overall, with only one individual with an index greater than zero (Figure 4.7a).

There was a statistically significant difference when all four cemeteries were compared together ( $p=0.033$ ). Post hoc testing showed that the significant difference existed between Lokomotiv individuals and Shamanka II individuals only (Figure 4.7b). Neither was significantly distinct from Ust'-Ida I or Khuzhir-Nuge XIV. Notably, Ust'-Ida I ranked second highest of the four cemeteries, which was the only time this occurred.

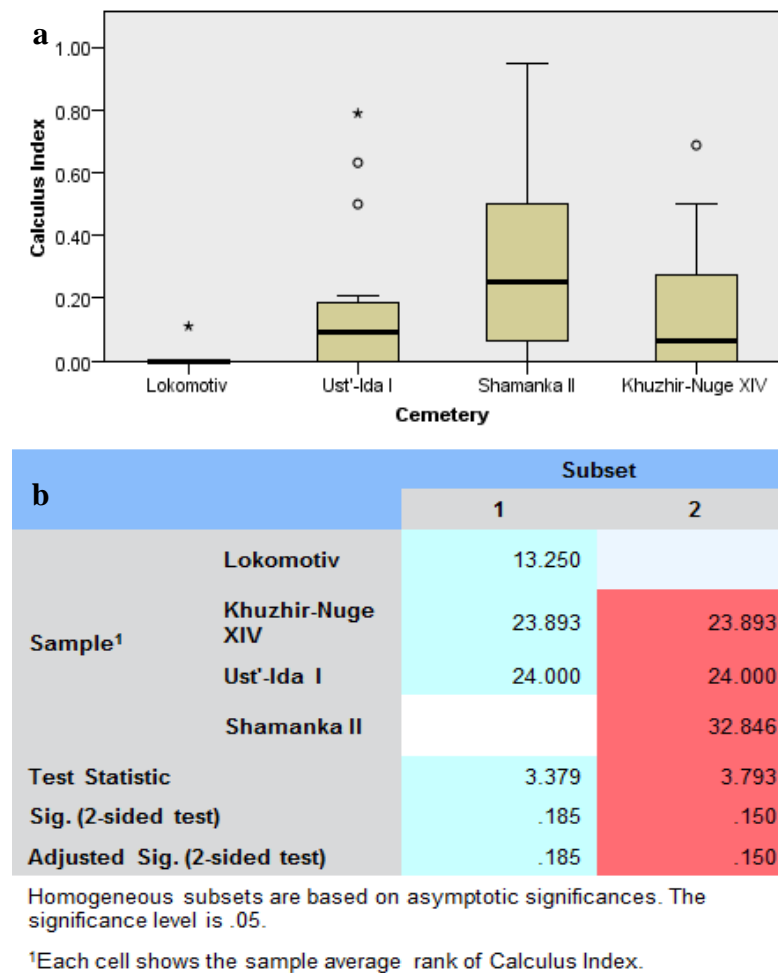


Figure 4.7 Results of analysis from LA calculus indices from juvenile individuals from all Cis-Baikal cemeteries: (a) Distribution of indices per cemetery; (b) Results of post hoc analysis.

#### 4.3.1.4 Lower Posterior Quadrant

Again, Shamanka II individuals exhibited the highest median score (0.143) and also the highest rank value from Kruskal-Wallis testing (Figure 4.8a, b; Table 4.2). Ust'-Ida I was ranked lowest, with only one individual with a score over zero.

Statistical analysis revealed that a significant difference existed among the cemeteries ( $p=0.004$ ). Ust'-Ida I individuals were significantly different from Khuzhir-Nuge XIV and Shamanka II ( $p<0.05$ ; Figure 4.8b). No significance was noted between Lokomotiv, Khuzhir-Nuge XIV, and Shamanka II individuals ( $p=0.158$ ; Figure 4.8b).

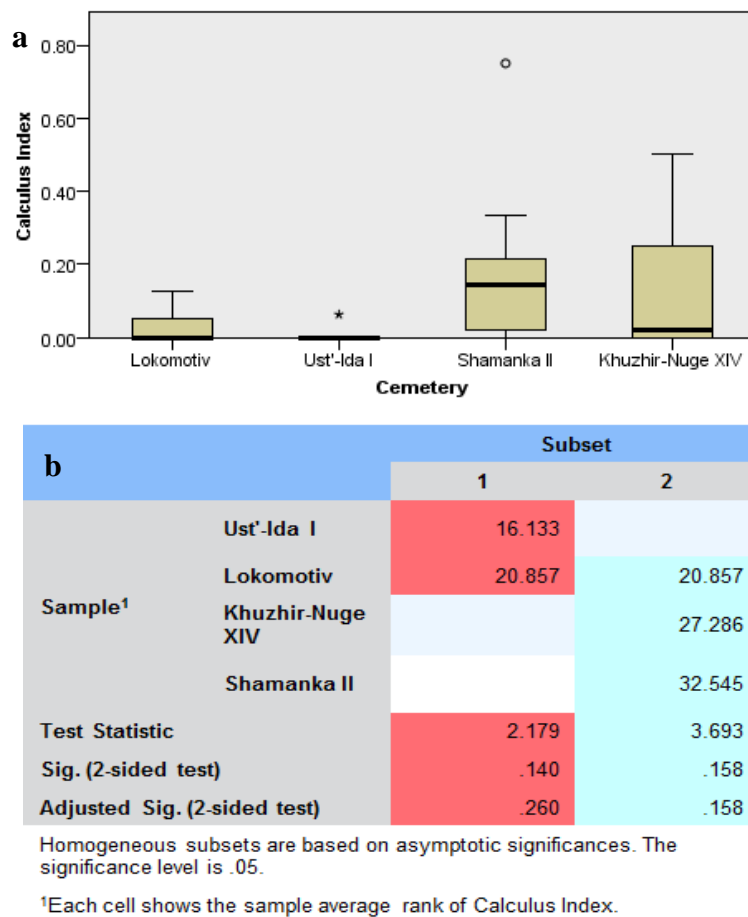


Figure 4.8 Results from analysis of LP calculus indices from juvenile individuals from all Cis-Baikal cemeteries: (a) Distribution of indices per cemetery; (b) Results of post hoc analysis.

#### 4.3.2 Adult Males

Kruskal-Wallis analysis and post hoc testing were conducted on each quadrant of the dentition for adult males. Poor preservation at Khuzhir-Nuge XIV and the lack of adults compared to juveniles at Ust'-Ida I resulted in small sample sizes for these two cemeteries (see Table 4.2). This likely decreased the ability to detect differences in the statistical analysis but unfortunately no other individuals were available to increase the sample size. Based on the results of the intrasite analysis, probable males were pooled with confidently identified males only for the UA and UP quadrants within Shamanka II and the UA and LP quadrants within Khuzhir-Nuge XIV, as no statistical significance was noted between these two groups. All other quadrants for Shamanka II and Khuzhir-Nuge XIV probable males had sample sizes less than three and were therefore not analyzed.

##### 4.3.2.1 Upper Anterior Quadrant

Shamanka II individuals exhibited the greatest calculus severity overall and highest rank while Ust'-Ida I displayed the lowest. A large outlier was present each from Lokomotiv and Ust'-Ida I; it was not removed as per Chapter 3, Section 3.1.3 (Figure 4.9a).

Kruskal-Wallis testing indicated that there was a significant difference among cemetery populations ( $p=0.002$ ) and post hoc testing was executed. The homogeneous subset table generated from stepwise step-down analysis showed that Ust'-Ida I, Khuzhir-Nuge XIV, and Lokomotiv were not statistically distinct from one another ( $p=0.570$ ). Shamanka II was separated into a subset of its own, indicating that calculus indices from Shamanka II were significantly different from all three other cemeteries (Figure 4.9b).

##### 4.3.2.2 Upper Posterior Quadrant

For the UP quadrant, Shamanka II and Khuzhir-Nuge XIV individuals had similar medians (0.345 and 0.273, respectively; Figure 4.10a; Table 4.2) and almost no difference in ranks ( $p=0.993$ ; Figure 4.10b). Ust'-Ida I had the lowest overall severity again. Individuals from Lokomotiv generally had quite low indices, though the individual associated with Burial L-16-1-1 had the largest deposits in the UP quadrant overall (Figure 4.10a). As mentioned in Chapter 3, Section 3.1.3, there was no justification for its removal and it, along with the two other outliers, were included within the population sample.

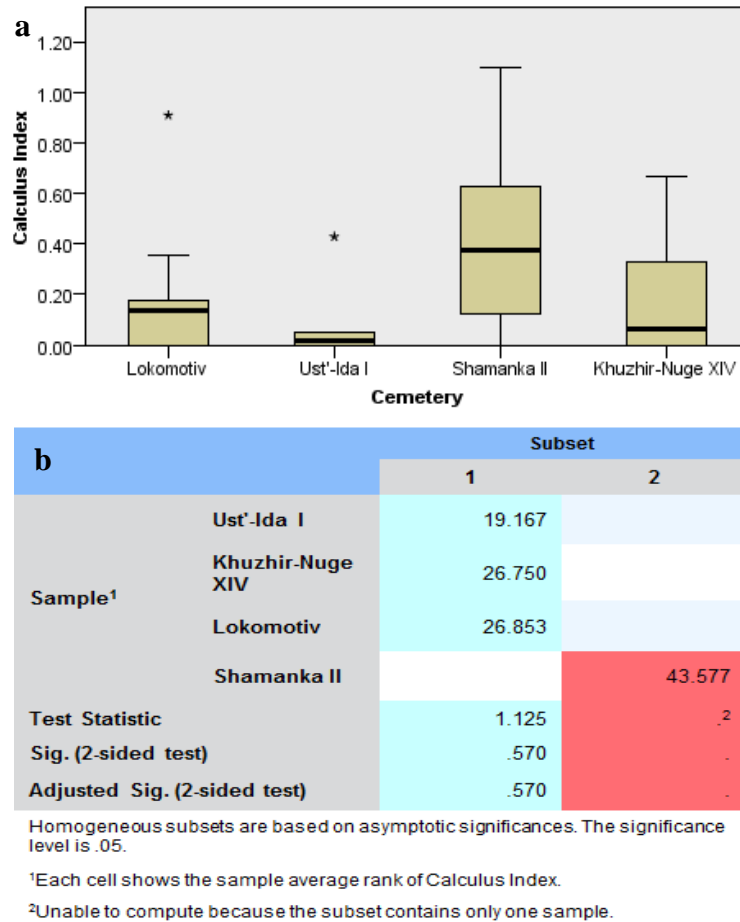


Figure 4.9 Results from analysis of UA calculus indices from adult male individuals from all Cis-Baikal cemeteries: (a) Distribution of indices per cemetery; (b) Results of post hoc analysis.

Significant differences were seen among the cemetery populations ( $p=0.000$ ). Shamanka II individuals exhibited significantly larger deposits than both Ust'-Ida I and Lokomotiv individuals ( $p<0.05$ ), but not when compared to Khuzhir-Nuge XIV. Ust'-Ida I and Khuzhir-Nuge XIV were also separated within separate subsets by post hoc testing, indicating statistical significance.

It was noted during the intrasite analysis that the UP calculus indices from Ust'-Ida I may be slightly problematic due to potential removal of deposits by post-excavation cleaning (see Section 4.1). The distribution (Figure 4.10a) was less extreme here when compared to Figure 4.2, perhaps because only males were present at this stage in the analysis; however, these results should be regarded cautiously.



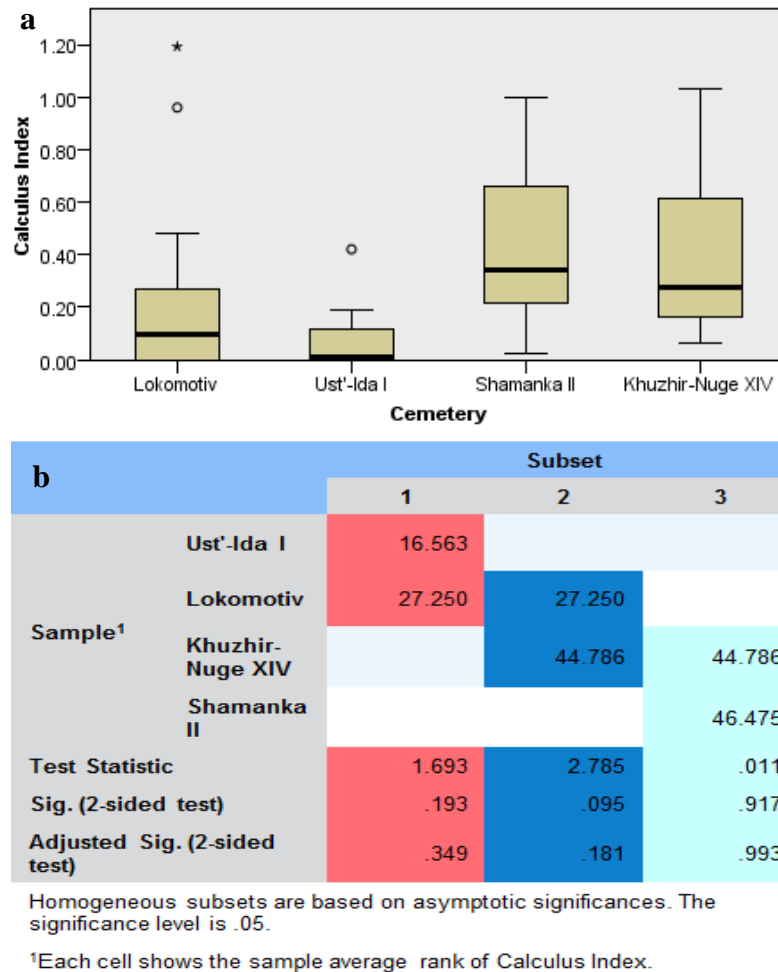


Figure 4.10 Results from analysis of UP calculus indices from adult male individuals from all Cis-Baikal cemeteries: (a) Distribution of indices per cemetery; (b) Results of post hoc analysis.

#### 4.3.2.3 Lower Anterior Quadrant

Shamanka II individuals exhibited the greatest severity overall (median=0.7105) while Ust'-Ida I individuals had the smallest indices (median=0.150), as well as much less variation in indices (Figure 4.11a).

Kruskal-Wallis analysis revealed distinctions among the cemetery populations ( $p=0.005$ ). Shamanka II individuals had significantly greater calculus severity than individuals from Lokomotiv and Ust'-Ida I. Surprisingly, although Khuzhir-Nuge XIV had lower values than Shamanka II individuals (Figure 4.11a) and a much lower median (0.308), no statistically significant difference was apparent in post hoc testing ( $p=0.445$ ). It should be noted that

Khuzhir-Nuge XIV had a sample size of only five, which may have limited the test's ability to distinguish a significant difference.

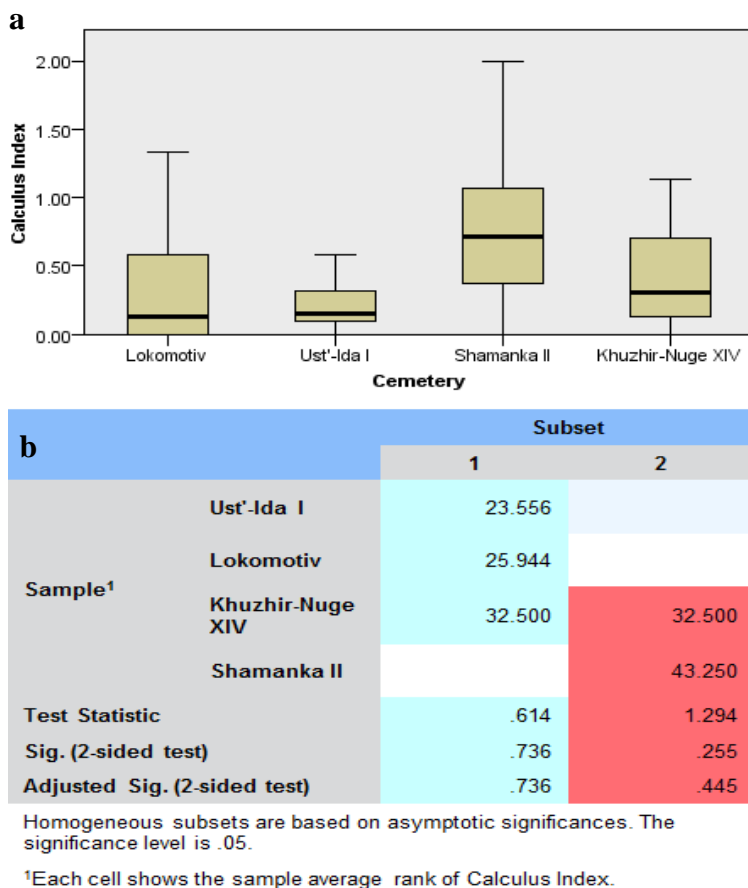


Figure 4.11 Results from analysis of LA calculus indices from adult male individuals from all Cis-Baikal cemeteries: (a) Distribution of indices per cemetery; (b) Results of post hoc analysis.

#### 4.3.2.4 Lower Posterior Quadrant

Shamanka II and Khuzhir-Nuge XIV individuals displayed similar calculus indices for the LP quadrants (Figure 4.12a; Table 4.2). Khuzhir-Nuge XIV actually had a slightly higher median than Shamanka II (0.556 versus 0.429), though Shamanka II was ranked higher in Kruskal-Wallis testing (Figure 4.12b). Following similar trends seen for previous quadrants, calculus indices from Ust'-Ida I were low with almost no variation in indices. Lokomotiv individuals exhibited intermediate indices within the data set.

Kruskal-Wallis tests revealed significant differences among the cemetery populations ( $p=0.006$ ). Stepwise step-down analysis clumped Ust'-Ida I and Lokomotiv together as being statistically similar and Lokomotiv, Khuzhir-Nuge XIV, and Shamanka II together (Figure 4.12b). As a result, differences were only noted between Shamanka II and Ust'-Ida I for the LP quadrant.

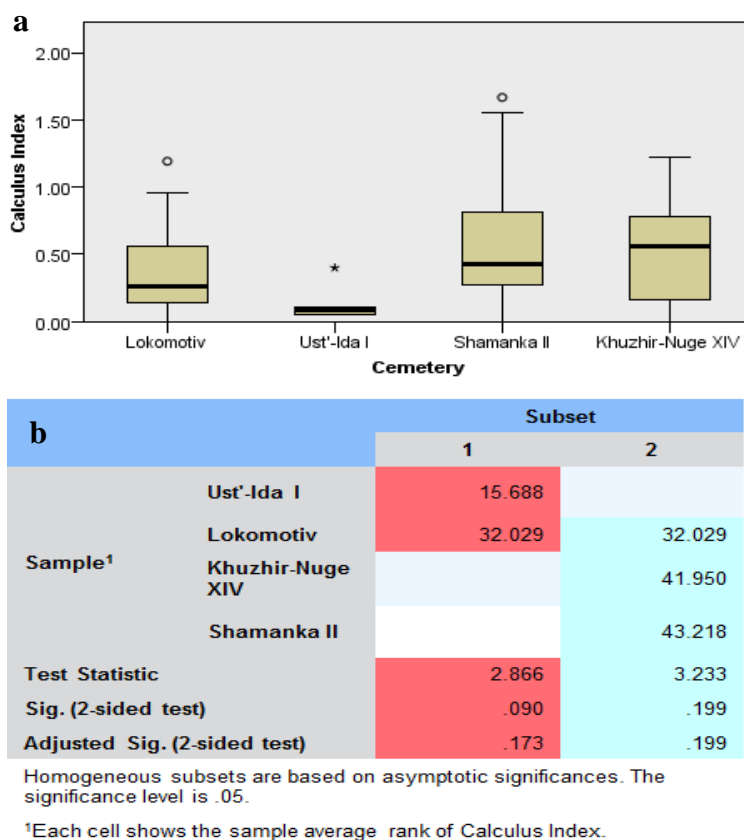


Figure 4.12 Results from analysis of LP calculus indices from adult male individuals from all Cis-Baikal cemeteries: (a) Distribution of indices per cemetery; (b) Results of post hoc analysis.

### 4.3.3 Adult Females

As mentioned in Section 4.2.4, sample sizes were too small for females from Khuzhir-Nuge XIV to be included in analyses. Therefore, intersite analyses consisted only of comparisons among Lokomotiv, Shamanka II, and Ust'-Ida I. Probable females were omitted from the Shamanka II sample since the intrasite analysis established that probable female sample sizes were too small for statistical analysis ( $n \leq 2$ ). The sample size for Ust'-Ida I females was small

(n=3). An overview of the results of Kruskal-Wallis tests for each quadrant is presented in Table 4.2.

#### 4.3.3.1 Upper Anterior Quadrant

Individuals from Shamanka II exhibited the greatest calculus severity overall (median=0.192), although the individual associated with Burial R-11-1 from Lokomotiv contained the actual highest score (outlier; Figure 4.13). Ust'-Ida I individuals had the lowest severity, with a median of 0.000. Kruskal-Wallis testing revealed that, despite these differences, no statistical significance was found among these cemeteries for adult females ( $p=0.122$ ).

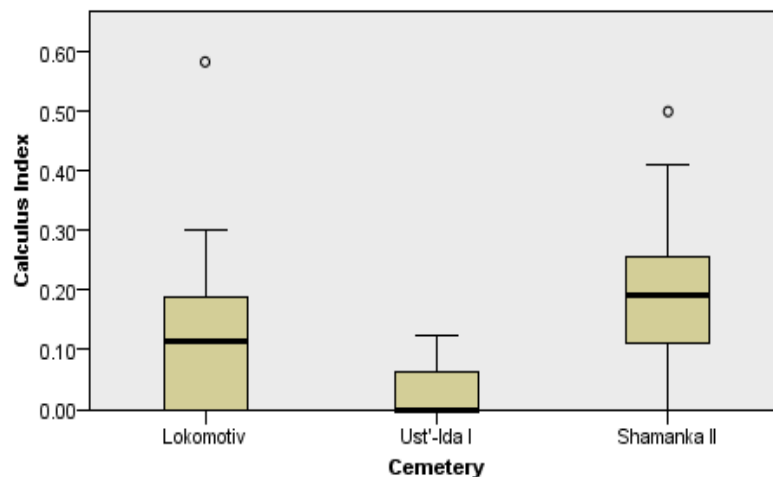
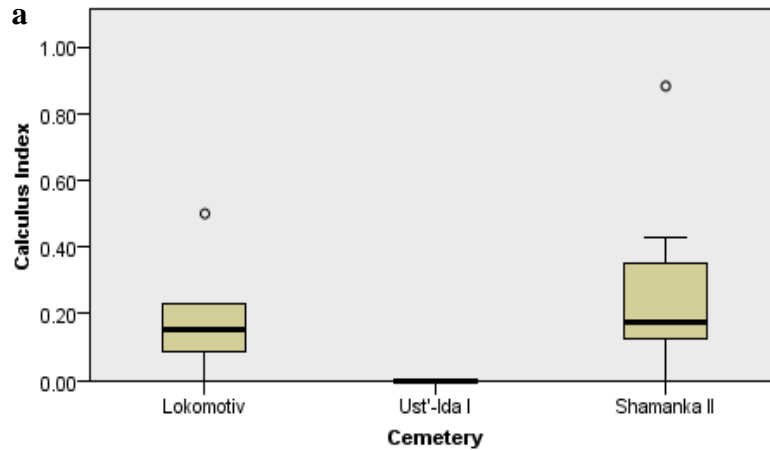


Figure 4.13 Distribution of the data for the UA quadrant from adult female individuals from Lokomotiv, Ust'-Ida I, and Shamanka II.

#### 4.3.3.2 Upper Posterior Quadrant

Females from Shamanka II and Lokomotiv exhibited similar calculus severity (Figure 4.14a), yet Shamanka II was ranked highest overall (Figure 4.14b). All individuals from Ust'-Ida I had no calculus present; therefore, it was ranked lowest.

Significant differences were found to exist among these cemetery populations ( $p=0.033$ ). Post hoc testing revealed that both Lokomotiv and Shamanka II had significantly higher calculus indices than Ust'-Ida I. Lokomotiv and Shamanka II were not statistically different from one another ( $p=0.495$ ; Figure 4.14b).



**b**

		Subset	
		1	2
Sample <sup>1</sup>	Ust'-Ida I	3.000	
	Lokomotiv		13.750
	Shamanka II		15.731
Test Statistic		. <sup>2</sup>	.466
Sig. (2-sided test)		.	.495
Adjusted Sig. (2-sided test)		.	.495

Homogeneous subsets are based on asymptotic significances. The significance level is .05.

<sup>1</sup>Each cell shows the sample average rank of Calculus Index.

<sup>2</sup>Unable to compute because the subset contains only one sample.

Figure 4.14 Results from analysis of UP calculus indices from adult female individuals from Lokomotiv, Ust'-Ida I, and Shamanka II: (a) Distribution of indices per cemetery; (b) Results of post hoc analysis.

#### 4.3.3.3 Lower Anterior Quadrant

Following the trends of the previous quadrants, adult females had the largest calculus deposits within the Shamanka II cemetery, while those from Ust'-Ida I had the smallest (Figure 4.15). However, no significant differences were seen among these populations during statistical analysis ( $p=0.370$ ).

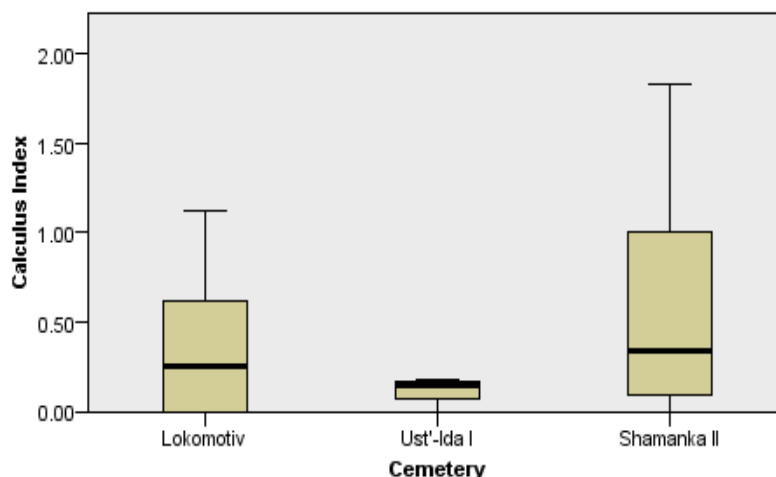


Figure 4.15 Distribution of the data for the LA quadrant from adult female individuals from Lokomotiv, Ust'-Ida I, and Shamanka II.

#### 4.3.3.4 Lower Posterior Quadrant

Ust'-Ida I appeared to have considerably lower calculus severity than Shamanka II individuals (median of 0.000 compared to 0.3535 respectively; Figure 4.16; Table 4.2), yet when all three cemeteries were compared through Kruskal-Wallis analysis, no significant difference was seen ( $p=0.133$ ). The small sample size for Ust'-Ida I ( $n=3$ ) may have had an effect on the outcome of analysis.

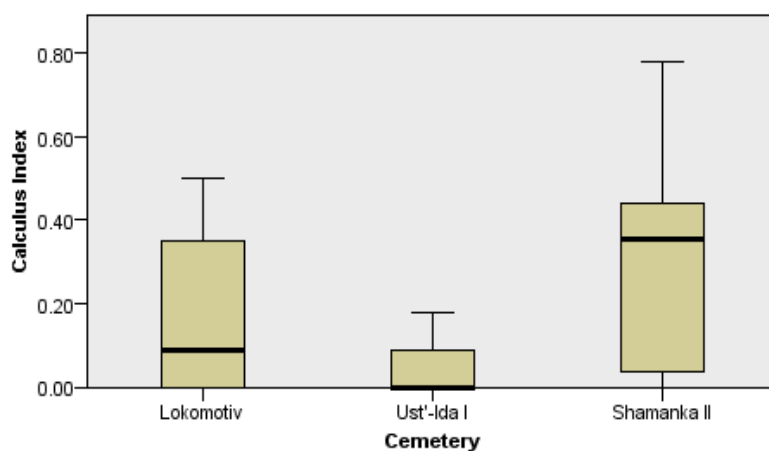


Figure 4.16 Distribution of the data for the LP quadrant from adult female individuals from Lokomotiv, Ust'-Ida I, and Shamanka II.

#### 4.3.4 Summary

For juveniles, Shamanka II individuals generally had the greatest calculus severity scores, but never significantly so. Interestingly, during stepwise step-down analysis, Shamanka II individuals were grouped with Khuzhir-Nuge XIV individuals for all four quadrants, while they were only grouped with Lokomotiv, the other EN Kitoi cemetery, for the UA and LP quadrants. The two LN-EBA ISG populations/samples, Khuzhir-Nuge XIV and Ust'-Ida I, were grouped together for anterior quadrants but not for posterior quadrants. In contrast, Ust'-Ida I and Lokomotiv were grouped together in every case.

Dental calculus severity in adult males presented a similar trend, with Shamanka II and Khuzhir-Nuge XIV grouped together for three out of four quadrants, though some of these results should be considered cautiously due to the large difference in sample size and preservation quality between the two populations. Ust'-Ida I and Lokomotiv were always grouped together, but Lokomotiv was also grouped with Khuzhir-Nuge XIV for all but the LP quadrant.

Shamanka II adult males had much larger calculus deposits in the two anterior quadrants of the dentition than did those from the other three cemeteries, though only the UA quadrant was significantly larger in statistical testing. In comparison, fewer differences were seen between Khuzhir-Nuge XIV, Lokomotiv, and Shamanka II in the posterior quadrants, but calculus indices for Shamanka II males were always significantly higher than those for Ust'-Ida I males. Similar to what was seen with juveniles; the two EN Kitoi populations were only grouped together for the LP quadrant while the two LN-EBA ISG populations were only grouped together for the UA and LA quadrant.

There were fewer differences among adult females when the cemeteries were compared. Shamanka II females exhibited significantly greater calculus severity than those from Ust'-Ida I in the UP quadrant but no other differences were noted in statistical testing for any other quadrant. It was unfortunate that sample size was too small to compare females from Khuzhir-Nuge XIV, as it would have likely shed additional light on important trends.

Altogether, the data suggested a general split between Shamanka II/Khuzhir-Nuge XIV and Ust'-Ida I/Lokomotiv, although Khuzhir-Nuge XIV was also similar to Lokomotiv in some cases. These trends were not distinct when comparing adult females from each population.

#### *4.3.5 Interpretation and Significance*

While sample size was likely an issue within some of the statistical tests, the results revealed some interesting trends in the data. If the dietary ratio of protein to carbohydrates alone was the primary factor influencing calculus severity, we would expect little to no significant severity differences among populations since both cultural groups were eating high protein/low carbohydrate diets, based on stable isotope analysis showing consumption of locally available fish and terrestrial game (Katzenberg and Weber 1999; Katzenberg et al. 2010; Weber and Bettinger 2010). Large severity scores were observed in all cemeteries, except possibly Ust'-Ida I, and most adults exhibited calculus to at least some extent in at least one quadrant, again except possibly at Ust'-Ida I, suggesting generally high rates of calculus formation consistent with a high protein/low carbohydrate diet. Yet, the results show that significant differences do exist both within and among Cis-Baikal populations. This indicates that the factors affecting calculus formation in the Cis-Baikal were complex and that we need to look beyond simple protein/carbohydrate ratios. Figure 2.1 presents a number of other factors that influence calculus formation, including stress, population affinity/genetics, environment, and non-dietary mouth use. Since an interpretation based purely on the proportion of protein and carbohydrates in the diet does not fit particularly well with the pattern of variation in the calculus results, these other factors were explored to see if they better explained the data.

Both population affinity and stress were likely not predominant factors in calculus formation within Cis-Baikal individuals. Mooder et al. (2006) examined mtDNA from individuals from both Lokomotiv and Ust'-Ida I and found that the haplogroup distributions for the two populations were significantly different, suggesting that the Kitoi and the ISG were biologically distinct. Yet, the results here show that Lokomotiv and Ust'-Ida I were often more similar to each other in terms of calculus severity than they were to their cultural counterpart populations, Shamanka II and Khuzhir-Nuge XIV, respectively. Shamanka II and Lokomotiv individuals were similar genetically (Mooder et al. 2010) but calculus severity determined from adult males was statistically different here.

Similarly, if stress was a predominant factor, EN Kitoi populations would be expected to be more similar to one another than to the LN-EBA ISG cemeteries. Prolonged breastfeeding was evident within EN subadults, and some infants died while still breast feeding, indicating possible periods of increased stress not seen in the LN-EBA (Waters-Rist et al. 2011). Statistical



analysis of dental calculus severity grouped Shamanka II and Khuzhir-Nuge XIV together more frequently and hence stress alone cannot explain the trends in the calculus data.

It is hypothesized here, following Lieverse et al. (2007a), that these similarities and differences in calculus indices may be the result of variances among geographical microregions and environments. Figure 1.2, in Chapter 1, shows a map of the Cis-Baikal region, separated into microregions. From this map, we can see that both Lokomotiv and Ust'-Ida I are located along the Angara riverine system, within the Angara River Valley microregion. Shamanka II and Khuzhir-Nuge XIV are both located along the shores of Lake Baikal, but within different microregions (South Baikal and the Little Sea microregion, respectively). As explained in Chapter 2, Section 1.2, as the Angara River moves away from Lake Baikal, tributary streams contribute more water, changing the ecology of the system (Weber 2003). It is very possible that environmental/resource differences between the riverine versus lake setting had an influence on calculus severity for the populations. Microregional differences between Cis-Baikal populations have been discerned in past BHAP research, including with regards to activity patterns, dental lesions, modifications, and accumulations, and stable isotope signatures (e.g., Lieverse et al. 2007a, 2013; Waters-Rist 2010; Katzenburg et al. 2010; Haverkort et al. 2010).

Another interesting result was that the differences noted between Lokomotiv and Shamanka II adult males were not also seen between their contemporary females. Stable carbon and nitrogen analysis has shown that signatures were more similar when Shamanka II and Lokomotiv were compared to each other, than when each was compared to other EN cemeteries (Katzenberg et al. 2010; Weber and Bettinger 2010). Weber and Bettinger (2010) state that while it is intuitively assumed that these two populations would exhibit larger differences in isotope signatures due to differences in local fisheries and their geographical separation, it is actually not that surprising that the two are similar compared to other EN population groups; they are both large cemetery populations that likely engaged in similar adaptive strategies and likely interacted. There also may have been some overlap in fisheries since EN populations were not completely sedentary (Weber and Bettinger 2010). These similarities may explain the lack of distinction between EN female groups in terms of calculus severity even though they are situated at home bases within varying microregions.

The distinctions between males but not females seem to enforce the notion that something is contributing to dental calculus accumulation in adult males at Shamanka II

specifically during the EN. It was suggested in Section 4.2.6 that the differences between males and females at Shamanka II may have been related to logistical foraging, which the EN Kitoi were thought to have engaged in more than the LN-EBA ISG (Weber et al. 2002). Also, although males engaged in frequent excursions for logistical foraging, the Kitoi as a whole are thought to have employed a lower residential mobility strategy involving extended stays at regionally restricted central bases along waterways from which these logistical excursions extended (Haverkort et al 2010; Lieverse et al. 2007b; Lieverse 2010; Weber et al. 2002; Weber and Bettinger 2010). While speculative, Shamanka II men may have spent time in territories completely different from both Shamanka II females and Lokomotiv individuals of both sexes and local factors in these new environments may have increased calculus accumulation. Water mineral content is a possible culprit, but specific resources such as seal, available only at Lake Baikal, may have contributed as well.

It has been well documented that archaeological Cis-Baikal populations situated on the shores of Lake Baikal engaged in seasonal seal (*Pusa sibirica*) hunting (Nomokonova et al. 2009; Weber et al. 1993, 1998). Stable isotope ratios suggest that populations in the Little Sea were utilizing seal to a greater extent than populations to the south (e.g., Shamanka II), but seal utilization has been detected in bone collagen from individuals in both areas (Katzenburg et al. 2010). Proteins, glycoproteins and lipids have been associated with the process of nucleation and initial calculus formation (Mandel and Eisenstein 1969; Slomiany et al. 1980). Slomiany et al. (1980) conducted studies on the lipid concentration in saliva between light versus heavy calculus formers and found that overall, heavy calculus formers' saliva contained 50 percent more lipids, by weight, than light calculus formers, with elevated levels of free fatty acids, cholesterol esters, and glyceroglycolipids. Both saliva and bacteria contribute to the lipid concentration in dental calculus (Slomiany et al. 1983). Though there is no available research on the effects of seal meat on dental calculus, it is possible that the lipids and proteins in seal meat may have affected the severity of calculus to some extent. This may have been a contributing factor to the distinction seen between populations situated around Lake Baikal, adjacent to seasonally available seal populations, and those in the Angara River Valley, far removed from them. If seal was hunted predominately by males, they would have had more immediate access to the resource as a dietary inclusion; this would explain the discrepancies between males and females in Shamanka II, due to greater social/gender differentiation among the Kitoi. Unfortunately, adult females were too

few at Khuzhir-Nuge XIV to contribute evidence on sex differences within the Little Sea microregion.

Other possible explanations for the patterns observed in Cis-Baikal adult males may be related to activity habits such as non-dietary mouth use rather than environment. Often anterior teeth are used as a tool (e.g., as a third hand) to assist in daily tasks. As mentioned previously, Waters-Rist et al (2010) noted occlusal grooves on the surfaces of teeth from Cis-Baikal individuals that were likely caused by running plant fibers or sinew through the teeth. Grooves were most prevalent on anterior teeth and within females. There was also a significant difference in the frequency of individuals with occlusal grooves between Angara River cemeteries (35 percent) and cemeteries situated on the lakeshore (13.3 percent). In comparison, calculus severity was generally higher in males and lakeshore populations, making it inversely correlated to occlusal grooves in that sense. Tool use may have removed calculus from tooth surfaces. In any event, it does not appear that this specific form of tool use was increasing calculus accumulation.

It is possible that mouth use activities other than fibre processing may have contributed to some of the differences noted among the sites. For males, there was a greater difference in calculus indices between Shamanka II and the other three cemeteries for anterior quadrants only. This increase in calculus may have been the result of male specific mouth use activities, particularly among Shamanka II individuals, that could have encouraged calculus accumulation on anterior teeth. For example, a long term population study on the natural course of calculus formation in Sri Lankan tea labourers found a correlation between greater calculus deposits and both smoking and betel chewing (Anerud et al. 1991). No evidence for this has been found archaeologically in the Cis-Baikal but the calculus data suggests that the mouth was likely used for a multitude of tasks that warrant further investigation. While speculating on specific mouth use activities is beyond the scope of this discussion, it is possible that these activities were related to logistical foraging and/or gendered social differentiation.

While these factors may help to explain the results of this study, other factors also need to be considered because they may have obscured the real quantity of calculus accumulation in the Cis-Baikal. These include individual variation, dental wear, and post-excavation removal of calculus.

While population affinity genetics did not seem to have played a large role, another genetic aspect may have been more important. In Chapter 2, it was noted that one of the factors

affecting calculus formation was simple individual variation, with some people deemed heavy calculus formers and others not (Mandel 1973, 1974). Figures 4.17 to 4.19 each display a graph of the relationship between calculus severity and age, with an interpolation line. It should be noted that these graphs are only approximations of the data. The data on age were determined as intervals originally but here they are displayed as the average of the upper and lower limits of the intervals. The figures show that, overall, the trends seen from statistical analysis are evident, but there are also large spikes and dips in the data that deviate from the trend. It is unlikely that individuals within a population ate drastically different diets or had great differences in mouth use patterns. It is much more likely that these represent heavy and light calculus formers. Due to small sample sizes, these were not averaged out within the dataset. Heavy and light calculus formers are natural occurrences in a population and therefore they should not be excluded, which is part of why outliers were not omitted from this study. Yet they also skew the general trend and need to be regarded cautiously.

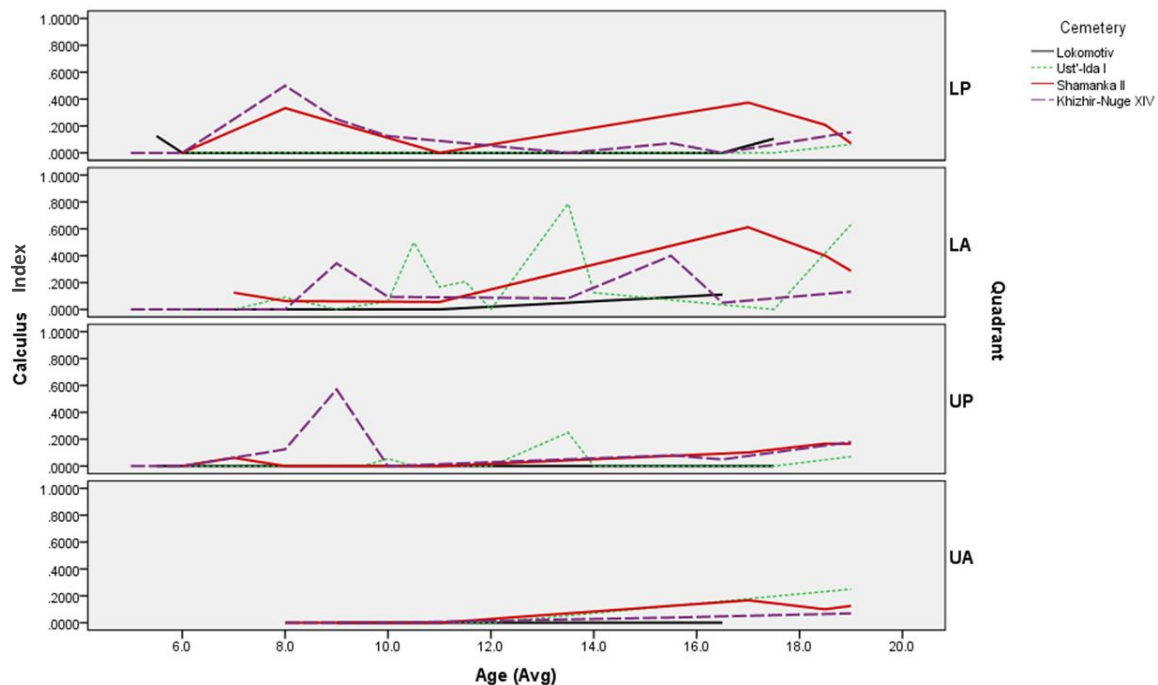


Figure 4.17 Interpolation graphs based on scatterplot data showing how the severity of calculus changes in relation to age within each quadrant of the mouth, for juveniles only.

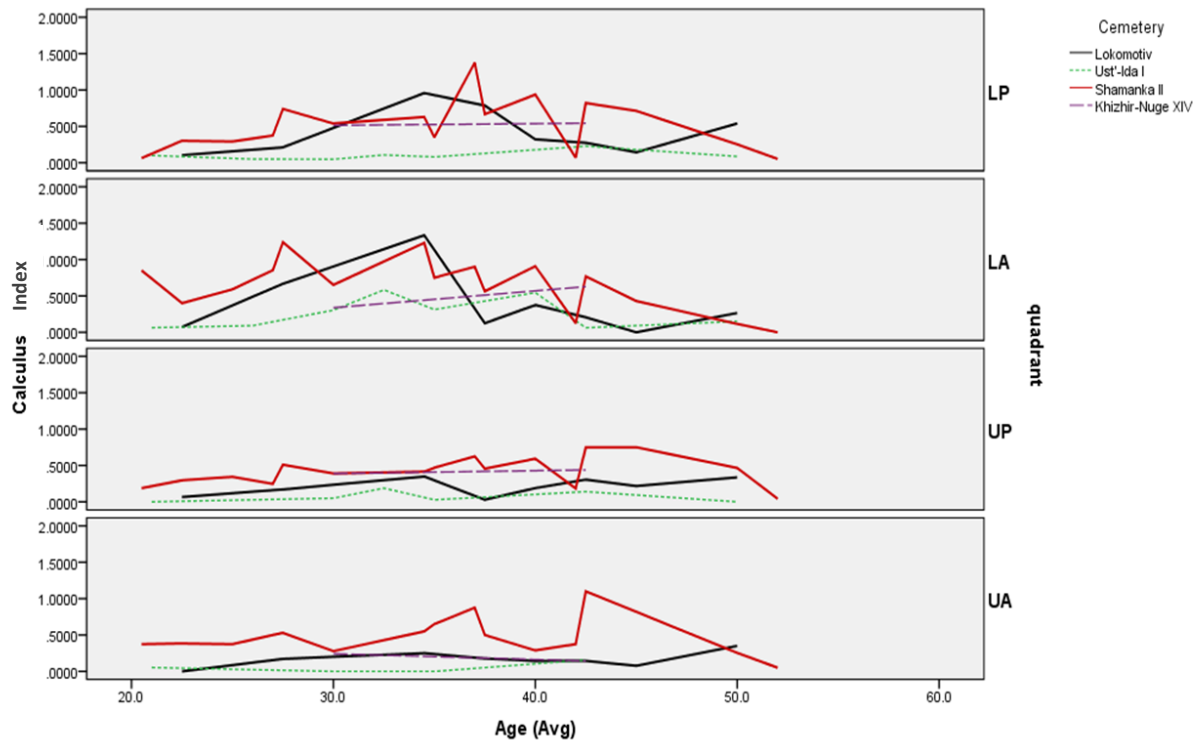


Figure 4.18 Interpolation graphs based on scatterplot data showing how the severity of calculus changes in relation to age within each quadrant of the mouth, adult males only.

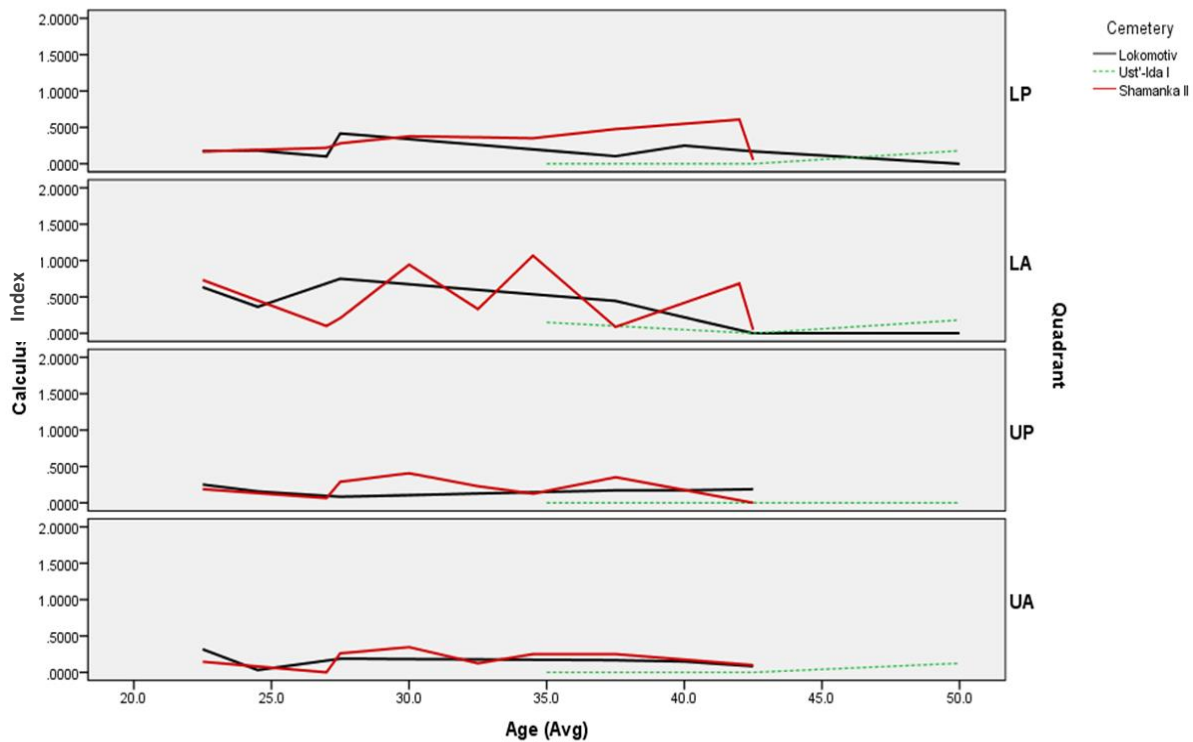


Figure 4.19 Interpolation graphs based on scatterplot data showing how the severity of calculus changes in relation to age within each quadrant of the mouth, adult females only.

It was already established that dental attrition was pronounced within the Cis-Baikal cemeteries (Lieverse et al. 2007a) and that it likely affected calculus severity of older adults. Figures 4.17 to 4.19 show the age-to-calculus relationships for juveniles, adult males, and adult females. Figure 4.17 clearly shows that calculus severity increased as individuals reached adulthood, yet Figures 4.18 and 4.19 show that, for the most part, calculus severity decreased as individuals reach 50 years of age. While severe dental wear patterns have provided other useful information about population dynamics, they diminished our ability to draw information from calculus accumulations in older adults.

Lastly, we cannot forget that at least some post-excavation removal of dental calculus had taken place, particularly for Lokomotiv and Ust'-Ida I. Removal of calculus before analysis may have contributed to the low severity documented for Lokomotiv and Ust'-Ida I. It is very likely that, with better preservation, curation, and knowledge of the utility of dental calculus, indices from both sites would have ranked at least slightly higher in analysis. However, it has been established that both cemeteries follow trends typical of calculus formation (Section 4.1); therefore enough calculus was left in situ to distinguish the patterns. The tendency of the data to lump Shamanka II/Khuzhir-Nuge XIV and Lokomotiv/Ust'-Ida I compares well with other research in the Cis-Baikal where microregional differences seem to override population affinity (e.g., Lieverse et al. 2007a, 2013, 2015; Waters-Rist 2010; Haverkort et al. 2010), but in this case the data also lumps the two cemeteries with likely postmortem loss. It is unfortunate that this bias towards more recently excavated cemeteries exists, but at the time Lokomotiv and Ust'-Ida I were excavated, dental calculus was considered a hindrance rather than a source of information. As more individuals and cemeteries are discovered in the Cis-Baikal, new information on calculus patterns will become available to expand on the interpretations drawn here.

Many potential causal factors have just been discussed and it would be advantageous to create an ordered system where these factors are ranked by the strength of their ability to influence calculus formation in the Cis-Baikal. Knowledge of strong causal factors within these populations could be used to build hypotheses for other research on dental calculus, both in the Cis-Baikal and outside, when environment, diet, mouth use activities, etc. are unknown. To this end, microregional environment or resource effects, such as differing water sources or access to specific resources such as seal, seem likely to have had the greatest influence on dental calculus formation in the Cis-Baikal, although post-excavation removal of calculus may have slightly

amplified this trend. Increasing age and associated increases in dental wear showed a strong negative correlation with calculus severity with increasing age suggesting that this is another main factor influencing the amount of calculus, although only in older adults. While dietary resources specific to microregions may have played a significant role, the general proportions of protein and carbohydrates in the diet seem to have had an observable but limited influence compared to the other factors discussed.

Yet, as outlined in Chapter 2, the aetiology behind calculus formation is complex and multicausal and it is therefore the opinion of the author that these factors do not fit well into a hierarchical or linear system and are generally situational to the group being studied. A calculus deposit is the end result all interacting factors and this is not straight forward. Factors may interact and/or mask one another. Hygiene or mouth use practices, and post-mortem removal, may remove the deposit eliminating all interpretive evidence. Individual variation may completely override any factor influencing calculus formation. The Cis-Baikal populations displayed many of these complicating factors. For example, while microregional factors may be a significant factor, Figures 4.17- 4.19 show that in some cases individual variation likely overrode all other factors regardless of their significance. Also, the specific influence of logistical foraging within microregional settings and possible mouth use activities are more speculative and it is unknown where these would fit within a hierarchical model but they fit well with the data trends.

#### **4.4 Chapter Summary**

Therefore, in the Cis-Baikal, dental calculus likely provides a loose proxy for diet, including the possible consumption of seal for populations situated around Lake Baikal. Calculus indices from Cis-Baikal individuals were often quite high, supporting assertions that these individuals had a high protein/low carbohydrate diet, but differences detected during statistical analysis also suggest that we need to look beyond simple dietary ratios to interpret calculus severity rates in the Cis-Baikal. Environmental differences and non-dietary mouth use are also factors known to influence calculus accumulation and, based on previous research by BHAP scholars on microregional differences, logistical foraging, and mobility strategies, these factors correlate well with the results presented here. This research has shown that the multifactorial nature of dental calculus formation, as well as obscuring factors such as attrition and post-excavation removal, make it quite difficult to pinpoint with certainty dietary causes of

differences in calculus accumulations and therefore all conclusions need to be carefully considered and supported by other lines of evidence, like those which will be explored in the Chapter 5.



## **Chapter 5**

### **Results and Interpretation of the Microscopic Analysis of Microparticles in Dental Calculus**

While research on dental calculus presence and severity has provided significant information on population lifeways, new research has begun to focus on the microscopic analysis of the deposits themselves. As outlined in Chapter 2, as plaque begins to mineralize into calculus, bacteria and debris from food and artifacts processed or manipulated with the teeth can become preserved in the matrix. The inclusion of debris in the matrix provides direct evidence of what the population was consuming or utilizing, since the mineralization process requires the presence of saliva, and therefore only takes place in vivo (Blatt et al. 2010). Calculus accumulates over an individual's life if not removed; therefore we may infer that at least several years of diet are represented in every individual studied (Henry and Piperno 2008). Also, because calculus is heavily mineralized, it survives well in archaeological contexts, preserving the microscopic remains encased within (Lieverse 1999). Here, calculus deposits from 10 individuals from each Cis-Baikal cemetery population were analyzed for the presence of microparticles that would have been incorporated in calculus during life. A variety of control samples were also examined for the presence of contaminants that may have obscured results. Combined, the results were used to determine the usefulness of this method for future research in the Cis-Baikal.

#### **5.1 Results of Control Sampling**

This results section begins with an outline of what was found within the three kinds of control samples used in this study to understand the environment in which samples were taken and processed. These were: 1) control swabs taken from storage enclosures, skeletal material/non-calculus-bearing parts of specimens from which calculus was collected, laboratory surfaces, and/or the researcher's hands; 2) particle traps set out to collect ambient contaminants in the Irkutsk State University and University of Saskatchewan laboratories while calculus collection and processing was taking place; and 3) blank controls comprised of empty microcentrifuge tubes that concurrently went through the same sets of microparticle extraction

steps as microcentrifuge tubes containing calculus samples. All particles found within calculus samples were compared to particles found within controls to help determine if they were modern or ancient in origin, since, especially while at the Irkutsk State University collecting samples, it was not possible to completely eliminate contaminants. Complete counts of all contaminants found within controls, as well as examples of what was found, are provided in Appendix D.

Originally, three to four controls were taken per individual during sampling at the Irkutsk State University: a mandible/maxilla swab from the skeleton sampled if available, a swab of the box or bag that the tooth was stored in, a particle trap set out for the duration of sampling, and sometimes a swab of the window ledge where sampling occurred or the researcher's hands (see Appendix C). Particle traps were usually left out for the duration of all sampling done within a single day; if multiple calculus samples were taken in the same day, the particle traps encompassed all those samples. Due to time constraints, only a subset of these Irkutsk State University laboratory particle traps and swab controls were scanned for contaminants. A total of 56 of 98 swab and particle trap controls taken while in the Irkutsk State University laboratory were chosen to create a reference collection of contaminants present at the time of sampling. These controls were chosen at random, for the most part, but some preference was given to controls associated with Cis-Baikal samples that yielded starch grains. All particle traps analyzed from the Irkutsk State University laboratory were negative for starch and phytoliths. Many of the skeletal element and container swab controls did contain starch grains though, with a few containing very large counts (e.g., Lokomotiv Burials L-15-1-1 and L-28-1-1; Appendix D, Table D-1; Appendix D, Figures D-2 to D-5). These were consistent to starch grains found in the study by Crowther et al. (2014) on contaminants within laboratory settings. Colored fibers were also fairly common in these controls. Small numbers of phytoliths, pollen, and spores (Appendix D, Figures D-1, D-6, and D-8) were also observed. These results are not surprising, considering the curation environment of the skeletal remains. For example, some of the boxes used for storing the remains were previously food boxes (Angela Lieveise, personal communication 2014). Also, many researchers have analyzed these remains in the past and, with so much contact, starch grain introduction was likely inevitable, especially in the absence of protocols designed to avoid transfer of residues from food, personal care products, etc.

Particle traps were left within the fumehood during an entire round of processing of samples at the University of Saskatchewan, an average of one to two days. During later rounds

particle traps were left out longer, roughly three days, over multiple processing rounds, as the process became more streamlined and continuous. Particle traps generally showed little to no signs of starch grain contamination (Appendix D, Table D-2). The only exception was particle trap seven, where five starch grains were found clumped together, varying in size but of similar shape and form otherwise. All had open and central hila and a roundish-polyhedral shape, consistent with the New World domesticate maize (*Zea mays*), which, by definition, would have to be a contaminant if observed in microparticle extractions from the Irkutsk State University calculus samples (Appendix D, Figure D-9b, c; Boyd et al. 2006; Boyd and Surette 2010). The plant particles from calculus samples processed while this particle trap was out were each compared closely with these contaminants, but no starch grains found within Cis-Baikal samples exhibited an open hilia. Therefore, while the particle trap confirms that some contaminant particles were airborne in the surrounding environment during the processing of these calculus samples, they do not appear to have entered the samples due to the stringent anti-contamination methods used during the microparticle extraction processing (see Chapter 3, Sections 3.2.3 and 3.2.4). A number of fibers were found within the particle traps as well, also suggesting some airborne contaminants, perhaps from clothing, were able to enter the fume hood, where almost all of the processing was done and where the particle traps were therefore laid.

One blank control was ran with every round of calculus samples during processing to control for particles that may have been introduced to the calculus samples through the solutions being used and to monitor if particles from the environment were getting into the samples despite the procedural measures used to minimize opportunities for contamination. For the most part, starch counts in these blank controls were between zero and two (14 of 16 rounds; Appendix D, Table D-3), which was acceptable, following Zarrillo (2012), who deemed a maximum of three acceptable in an older laboratory facility comparable to that at the University of Saskatchewan. A complete lack of starch grains would have been ideal but, without a clean room with positive pressure, this was likely unattainable. With this in mind, only two blank controls were considered problematic. Seven starch grains, with varying characteristics, were observed in blank control one (Appendix D, Table D-3 and Figure D-10). As discussed in Chapter 3, Section 3.2.4, methods and protocols were reassessed due to this contamination, with more stringent measures applied during subsequent sample processing. Blank control 14 contained five starch grains as well, even though it was processed after the new methods and protocols were found to correct

the issues observed in blank control one (Appendix D, Table D-3 and Figure D-10); this appeared to be due to an anomalous contamination incident of unknown source, and therefore the starch grains from calculus samples associated with this control were treated with extra caution.

Overall, it can be seen that starch contamination was a concern while sampling at Irkutsk State University, but, as will be seen, starch counts within the actual calculus samples were much lower than in many of the associated controls, showing that sample processing and contamination protocols were effective in preventing sources of starch contamination at the Irkutsk State University facility from impacting the calculus samples. Starch contamination in the laboratory at the University of Saskatchewan was present but managed by careful analysis of controls and adjustment of procedures, again successfully minimizing potential impacts to the calculus samples.

## **5.2 Cis-Baikal Sample Results**

Table 5.1 provides an overview of the microparticles found within each calculus sample analyzed. In total, 31.1 percent (14/45) of samples were taken from anterior teeth, with deposits ranging from 0.6 mg to 4.6 mg and averaging 1.6 mg. In contrast, 68.9 percent (31/45) were from posterior teeth, with deposits ranging from 0.1 mg to 25.2 mg and averaging 4.8 mg. The largest deposits mainly came from Shamanka II individuals, with an average deposit of 7.2 mg, while Ust'-Ida I generally had the smallest deposits, with an average of 1.7 mg. The average was 2.8 mg for Lokomotiv and 2.3 mg for Khuzhir-Nuge XIV. Whereas the researcher was primarily looking for starch and phytoliths within the sample, a variety of additional particles were noted as well. A full synopsis of what was found within each sample is provided in Appendix E.

### *5.2.1 Contamination within Samples*

Despite all procedural controls in place, a small amount of contamination was suspected to be present in a number of calculus samples. This included a number of starch grains found to match those recovered from swabs and blank controls, a variety of fibers from the external environment, and lacquer fragments.

Eighteen starch grains found within Cis-Baikal calculus samples were found to closely resemble starch grains found in controls (Figure 5.1; Table 5.1). This did not necessarily dictate that they were contaminants, except in cases where the particles were from sources unavailable to Neolithic Siberian populations (e.g., maize starch). Still, starch from plant species consumed

Table 5.1 Results of Microscopic Analysis of Dental Calculus Samples

Cemetery	Burial*	Tooth	Weight (mg)	Starch (non-contam.)	Starch (contam.)	Phytolith	Pollen	Spores/Fungal Activity	Fibers (contam.)	Other
Lokomotiv	R-14-1	35**	0.1	0	0	0			Yes	
Lokomotiv	L-38-2-1	43**	1.9	0	1	0				
Lokomotiv	L-28-1-1	46	1.6	0	1	0		Yes		
Lokomotiv	L-28-1-1	41	0.6	2	0			Yes		
Lokomotiv	L-15-1-1	31**	1.8	2	0	0				
Lokomotiv	R-11-1	23	1.5	0	0	3?		Yes		
Lokomotiv	L-35-1-1	31	1.0	1	0	0	Yes	Yes		
Lokomotiv	L-20-2-1	18	14	2+3?	0	0			Yes	Translucent minerals with protrusions, bacterial chain, unknown particle with stem
Lokomotiv	L-16-1-1	45	4.6	0	0	0		Yes	Yes	
Lokomotiv	R-15	36	2.9	1	0	1?				Rod-shaped particle with scalloped edges
Lokomotiv	L-9-1-1	15	0.8	4	0	2+1?		Yes		Cell clump, likely bacterial, unknown opaque particle, plant fragment
Shamanka II	47-1	41	2.3	0	1	0		Yes		
Shamanka II	47-1	36	8.0	3	0	0		Yes		
Shamanka II	30-1	46	14.7	1	0	0		Yes		Translucent unknown tissue
Shamanka II	30-1	46	25.2	2	0	1	Yes	Yes		Testate amoeba, hair-like particle
Shamanka II	60-2	44	3.6	2	0	1?				
Shamanka II	46-1	15	2.2	2	1?	0			Yes	Unknown particle with protrusions
Shamanka II	46-1	46	14.2	0	1	0	Probable	Yes		
Shamanka II	24	18	4.3	1	0	0				
Shamanka II	76-1	43	4.6	1?	0	0			Yes	
Shamanka II	66-1	41	1.8	1	1	0		Yes		
Shamanka II	26-3	85	1.5	2	0	0		Yes		

Cemetery	Burial*	Tooth	Weight (mg)	Starch (non- contam.)	Starch (contam.)	Phytolith	Pollen	Spores/ Fungal Activity	Fibers (contam.)	Other
Shamanka II	109-1	26	13.5	0	0	0		Yes		Possibly diatom
Shamanka II	67	75	0.6	2	0	0		Yes	Yes	
Ust-Ida I	14-1-1	41	0.6	0	0	0				
Ust-Ida I	6-1	15	0.9	2	0	0			Yes	
Ust-Ida I	38-1-1	32	2.3	1	1	0	Yes	Yes	Yes	
Ust-Ida I	45-1-1	32	1.2	1	0	0		Yes	Yes	
Ust-Ida I	33-1-1	25	2.9	1	0	0			Yes	
Ust-Ida I	44-2	85	0.6	0	0	0				
Ust-Ida I	19-1	45	0.8	0	0	0				
Ust-Ida I	5-1-1	42	1.0	0	0	0				
Ust-Ida I	56-1	17	4.2	1+1?	1	1-2?			Yes	
Ust-Ida I	30-1-1	45	2.6	0	2	0		Yes		
Khuzhir-Nuge XIV	35-2	26**	3.2	0	0	0				Charcoal fragments, possible plant particle fragment
Khuzhir-Nuge XIV	52	45**	8.0	1	0	1?		Yes		Charcoal fragments
Khuzhir-Nuge XIV	27-1	26**	1.0	1	0	3-4?		Yes		Bacterial chains
Khuzhir-Nuge XIV	37-2	41	0.6	0	1?	0		Yes		
Khuzhir-Nuge XIV	33	55	0.2	0	0	0		Yes		
Khuzhir-Nuge XIV	12	15	0.7	2	1	0		Yes		
Khuzhir-Nuge XIV	12	15 (2nd piece)	1.0	0	1?	0		Yes		Bacterial chains
Khuzhir-Nuge XIV	35-1	11**	0.6	1	2	0		Yes	Yes	
Khuzhir-Nuge XIV	34	35	1.0	0	1?	0	Probable	Yes		
Khuzhir-Nuge XIV	32	27	1.6	2	1	0		Yes		Charcoal fragment

Cemetery	Burial*	Tooth	Weight (mg)	Starch (non- contam.)	Starch (contam.)	Phytolith	Pollen	Spores/ Fungal Activity	Fibers (contam.)	Other
Khuzhir-Nuge XIV	11	47	7.2	2+1?	1	0		Yes		

contam.= contaminant; \* when burial is listed twice the first listed is sample one and the second is sample two; \*\* two subsamples taken; ?=probable

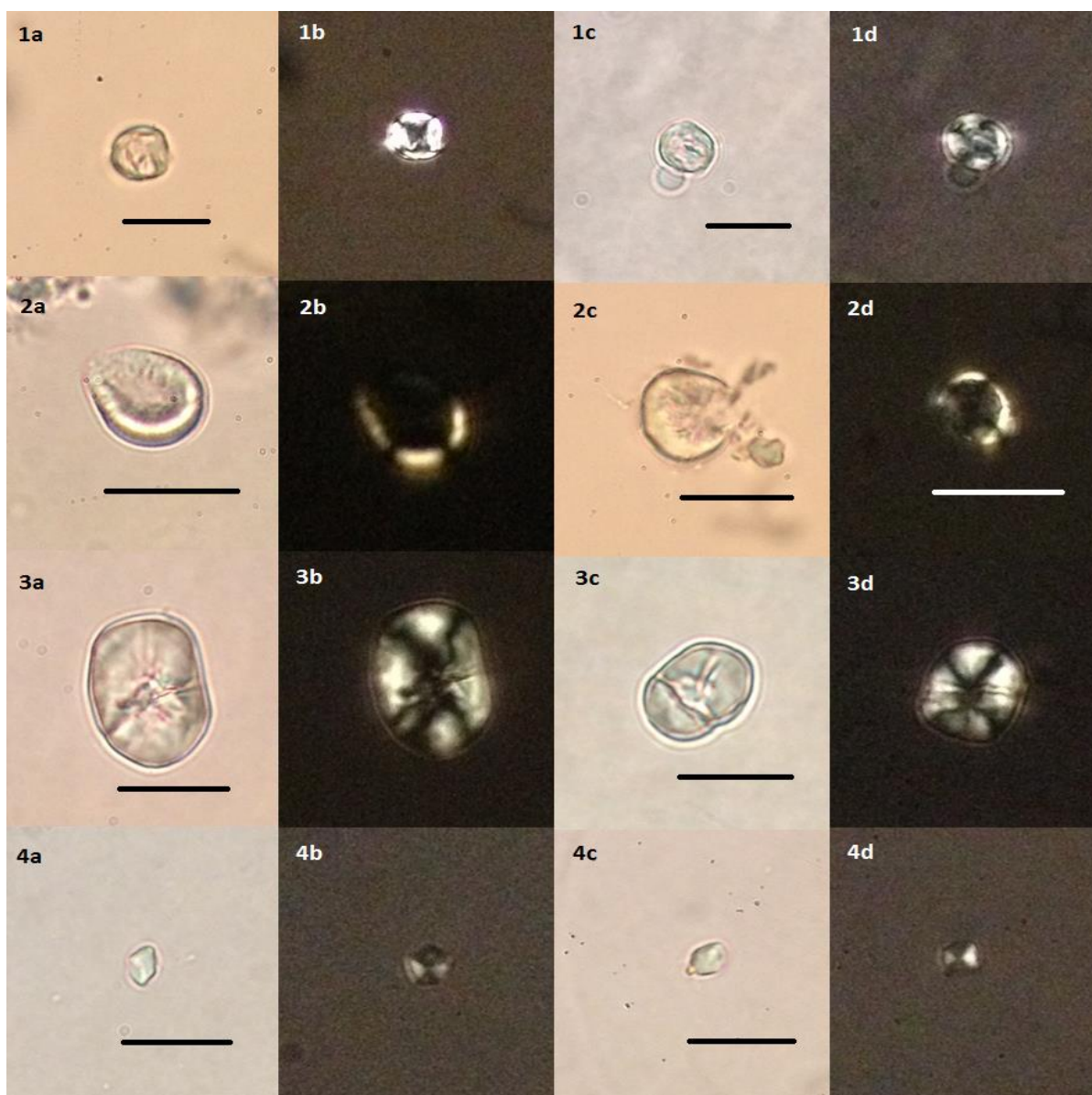


Figure 5.1 Examples of starch grains recovered from Cis-Baikal samples and corresponding contaminants from controls, a, c=normal light, b, d=cross polarized light, scale=15  $\mu$ m: (1a, b) Lokomotiv Burial L-28-1-1, sample two starch grain compared to (1c, d) Lokomotiv Burial L-28-1-1 mandibular swab contaminant starch grain; (2a, b) Shamanka II Burial 47-1, sample one starch grain compared to (2c) Shamanka II Burial 47-1 bag swab starch grain and (2d) Shamanka II Burial 47-1 mandible swab starch grain; (3a, b) Shamanka II Burial 66-1 starch grain compared to (3c, d) Blank control 12 starch grain; (4a, b) Khuzhir-Nuge XIV Burial 11 starch grain compared to (4c, d) Ust'-Ida I Burial 14-1-1 mandible swab starch grain.

by eastern Siberian middle Holocene hunter-gatherers can reasonably be assumed to have been quite different than typical domestic plant starch contaminants found in a laboratory setting. In



order to provide the most rigorous analysis, they were deemed to be contaminants and were removed from further consideration as a precaution. Most were found to match swab controls rather than blank controls, suggesting that most of the contaminants adhered to the calculus prior to sampling in the Irkutsk State University laboratory and minimal contamination occurred within the University of Saskatchewan laboratory. It was unfortunate to remove potential data but stringent precautionary measures needed to be taken due to the high levels of contamination seen in many controls from the Irkutsk State University laboratory. Consequently, only starch grains adequately distinct from controls, based on the researcher's judgement, were confidently associated with in vivo incorporation within calculus.

Contaminant fibers were also found in 12 subsamples. Eight of these 12 subsamples were associated with individuals from Lokomotiv and Ust'-Ida I, which were excavated about 20 years before the other two cemeteries and had therefore been curated for much longer. Fibers found included two stiff synthetic fibers, one red or pink and one whitish-opaque to translucent, a variety of blue clothing fibers, a striated clear fiber of unknown origin, and a large number of uncoloured cellulose fibers (Figure 5.2); an indigo particulate that was likely associated with denim apparel was also observed. As mentioned in Chapter 3, Section 3.2.4, many of the cellulose fibers were thought to have originated from the autoclave bags, within which the slides were sterilized, as a macroscopically visible residue was seen on most slides once removed from the bags. Similar cellulose fibers were microscopically observed on blank autoclaved slides, supporting this conclusion. These were not included within the "Fiber" column of Table 5.1, since they were fairly ubiquitous within each subsample and their origins were known.

It was noted in the Irkutsk State University facility that many individuals from Lokomotiv and Ust'-Ida I had lacquer adhering to the surface of skeletal elements, and as a consequence, to their calculus deposits as well. This is typically done to preserve and protect remains from post-excavation damage. Lokomotiv Burial L-20-2-1 was one such case, and the researcher attempted to abrade the surface to remove the lacquer prior to sampling. Despite this, the researcher found large particles of a solid, transparent to opaque film most likely to have been fragments of lacquer within the calculus subsample analyzed (Figure 5.3a). All starch grains from the subsample were considered distinct enough from contaminants in the associated controls to be kept in the analysis, but the lacquer may have introduced some contamination,



Figure 5.2 Sample of contaminant fibers recovered from Cis-Baikal calculus samples, scale=100  $\mu\text{m}$ : (a) Indigo particulate from Lokomotiv Burial R-15-1; (b,c) Blue clothing fibers from Lokomotiv Burial L-16-1-1 and Khuzhir-Nuge XIV Burial 35-1, sample two, respectively; (d) Blue cellulose fiber from Khuzhir-Nuge XIV Burial 35-1, sample one; (e) Unknown striated fiber from Lokomotiv Burial L-16-1-1; (f) Red/pink synthetic fiber in Ust'-Ida I Burial 6-1; (g,h) Clear synthetic fiber from Ust'-Ida I Burial 38-1-1; (i-l) Cellulose fibers from Ust'-Ida I Burial 19-1, Lokomotiv Burial L-20-2-1, Khuzhir-Nuge XIV Burial 11, and Ust'-Ida I Burial 19-1, respectively.

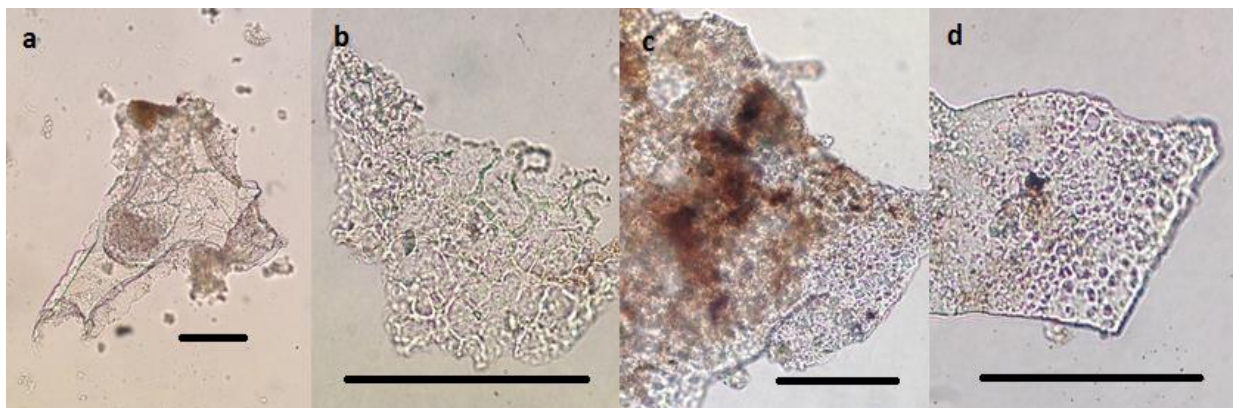


Figure 5.3 Lacquer fragments recovered from Cis-Baikal calculus samples, scale=100  $\mu\text{m}$ : (a) Lokomotiv Burial L-20-2-1; (b) Lokomotiv Burial L-9-1-1; (c,d) Ust'-Ida I Burial 5-1-1.

including blue fibers seen in the subsample. Two other subsamples, from Lokomotiv Burial L-9-1-1 and Ust'-Ida I Burial 5-1, exhibited similar thin, opaque solid fragments, again probably from lacquer applied to the skeletons (Figure 5.3b-d). No lacquer was noted on the surface while either sample was removed from the tooth surface. For Ust'-Ida I Burial 5-1, observation of the

calculus sample extract under magnification showed that calculus material remained adhered within and to this lacquer material, even after treatment with HCl (Figure 5.3c). No plant particles were found within this sample and it may have been due to the fact that, unlike the other calculus samples, the lacquer prevented the microparticle extraction process from disaggregating the calculus from this individual.

### 5.2.2 *Starch grains*

A total of 67 starch grains and possible starch grains were found within calculus subsamples from Cis-Baikal individuals (Table 5.1). As mentioned, 18 of these were removed from the analysis. Consequently, 49 starch grains were considered to be distinct enough from control contaminants to be confidently considered ancient in origin: 15 from Lokomotiv, 17 from Shamanka II, 7 from Ust'-Ida I, and 10 from Khuzhir-Nuge XIV. Sample size was not necessarily a large factor in plant particle recovery. For instance, Shamanka II Burial 109-1 had a sample weighing 13.5 mg that produced no starch grains within its subsample, while Lokomotiv Burial 9-1-1's sample was much smaller (0.8 mg) and had a relatively large starch grain count of four within its subsample.

Notably distinct starch grains were found in Khuzhir-Nuge XIV Burials 11 and 52, Ust'-Ida I Burials 56-1 and 33-1-1, Shamanka II Burials 67, 24, 30-1 and 47-1 (Figure 5.4). Others more closely resembled starch grains seen in controls but had at least one major distinguishing feature (e.g., birefringence and/or extinction cross characteristics, size). A summary of key characteristics of each of these starch grains is provided in Table 5.2. Based on their diversity, these starch grains appeared to represent a variety of plant species, with no type dominating the assemblage. In many cases, observed starch grains were exceptionally small and generally featureless, even when observed with the highest power of the microscope (600X). They are likely not identifiable to a particular species and some may even represent transient starch grains. Unfortunately, similar featureless tiny starch grains appeared in some control samples, which presented a dilemma about whether all of these were best discarded from the analysis. It was unfortunate to remove them from analysis when the only feature connecting many of them was their size and general lack of features but, to maintain rigour, it was necessary. The only exception was when they were found to be directly associated with calculus material. Some of the starch grains identified were found firmly attached to or embedded within undissolved calculus within the slide-mounted extracts. These could not be dislodged even with heavy

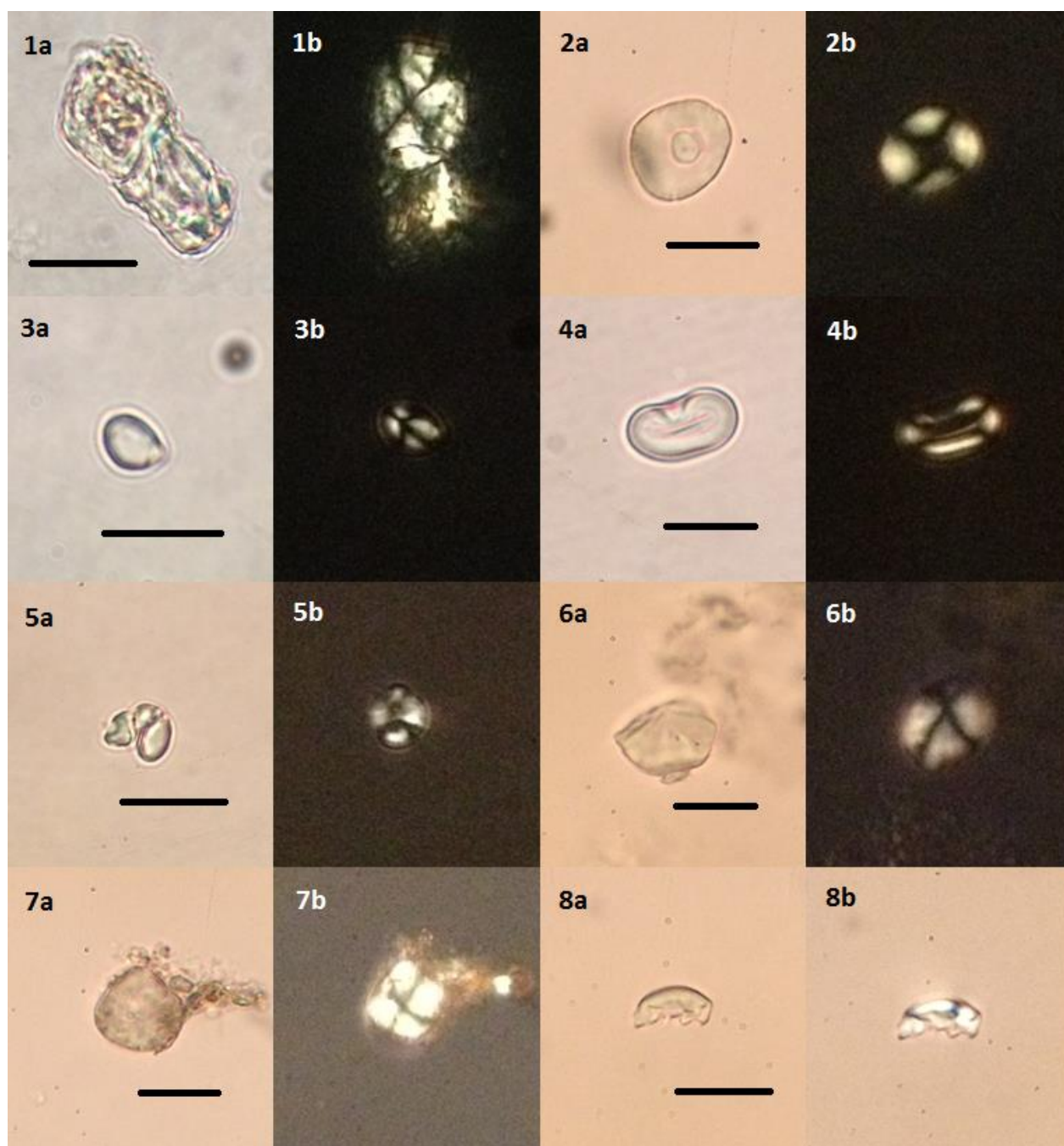


Figure 5.4 Examples of distinct starch grains recovered from calculus associated with Cis-Baikal individuals, a=normal light, b=cross polarized light, scale=15  $\mu$ m: (1a, b) Khuzhir-Nuge XIV Burial 11, sample one; (2a, b) Khuzhir-Nuge XIV Burial 52, subsample two; (3a, b) Ust'-Ida I Burial 56-1; (4a, b) Ust'-Ida I Burial 33-1-1; (5a, b) Shamanka II Burial 67; (6a, b) Shamanka II Burial 30-1, sample one; (7a, b) Shamanka II Burial 24; (8a, b) Shamanka II Burial 47-1, sample two.

Table 5.2 Summary of Key Characteristics for Identified Starch Grains from Cis-Baikal Calculus Samples

Cemetery	Burial	Shape*	Size (µm)**	Hilum (open or close/central or acentric)	Lamellae (present or absent)	Fissures (present or absent/type)	Birefringence	Damage	Other/Notes***
Lokomotiv	L-28-1-1	Round	4.7	Closed/Central	Absent	Absent	Cross arms at right angles	None	Indentation at hilum; could not rotate grain to definitively determine shape; from sample one
Lokomotiv	L-28-1-1	Round	3.6	Closed/Central	Absent	Absent	Cross arms at right angles, cross weak	None	Indentation at hilum; could not rotate grain to definitively determine shape; from sample one
Lokomotiv	L-15-1-1	Roundish	~3-4	Closed/Unknown	Absent	Absent	Weak birefringence	None	Attached to another particle; too small of distinguish most features; from subsample two
Lokomotiv	L-15-1-1	Roundish	~3-4	Closed/Central	Absent	Absent	Weak extinction cross with arms at right angles	None	Attached to demineralized calculus; too small to distinguish most features; from subsample two
Lokomotiv	L-35-1-1	Concave-lenticular	23.1	Closed/Central	Present	Absent	Cross arms wide and not connected in middle when rotated on side	None	Lenticular starch grains were observed in controls but none matching both the concavity and extinction cross
Lokomotiv	L-20-2-1	Oval	18.7	Closed/Central	Absent	Absent	Strong birefringence; cross arms at right angles	None	Slight depression in middle of grain
Lokomotiv	L-20-2-1	Convex-lenticular	24.3	Closed/Central	Present	Absent	Cross arms wide and barely connected in middle	None	
Lokomotiv	L-20-2-1	Round	4.9	Closed/Central	Absent	Absent	Very faint	None	Attached to demineralized calculus
Lokomotiv	L-20-2-1	Unknown	<5	Unknown	Unknown	Unknown	Very faint	Unknown	Only identified as possible starch; cannot see grain in normal light; embedded deep within calculus

Cemetery	Burial	Shape*	Size ( $\mu\text{m}$ )**	Hilum (open or close/central or acentric)	Lamellae (present or absent)	Fissures (present or absent/type)	Birefringence	Damage	Other/Notes***
Lokomotiv	L-20-2-1	Unknown	<5	Unknown	Unknown	Unknown	Very faint	Unknown	Only identified as possible starch; cannot see grain in normal light; embedded deep within calculus
Lokomotiv	R-15-1	Rounded pyramidal/ semi-circular	12	Closed/Central	Absent	Absent	Cross arms sharp and slightly curved	None	Faceted bottom; should be considered cautiously due to association with contaminated blank control 14
Lokomotiv	L-9-1-1	Convex-lenticular	25.5	Closed/Central	Present	Absent	Cross arms wide and dull, when rotated a horizontal bar appeared to connect the two arms of the grain in the middle	None	Protuberance at one end of grain; should be considered cautiously due to association with contaminated blank control 14
Lokomotiv	L-9-1-1	Likely roundish/ polyhedral	4.6	Closed/Central	Absent	Absent	Faint birefringence, H-shaped extinction cross	None	Could not rotate to definitively determine shape; indentation at hilum; should be considered cautiously due to association with contaminated blank control 14
Lokomotiv	L-9-1-1	Likely round	5.6	Closed/Central	Absent	Absent	Faint birefringence, H-shaped extinction cross	None	Attached to demineralized calculus, could not rotate to definitively determine shape; indentation at hilum; should be considered cautiously due to association with contaminated blank control 14
Lokomotiv	L-9-1-1	Likely polyhedral	9.5	Closed/Central	Absent	Absent	Faint birefringence, H-shaped extinction cross	None	Could not rotate to definitively determine shape; indentation at hilum; should be considered cautiously due to association with

Cemetery	Burial	Shape*	Size ( $\mu\text{m}$ )**	Hilum (open or close/central or acentric)	Lamellae (present or absent)	Fissures (present or absent/type)	Birefringence	Damage	Other/Notes***
Shamanka II	47-1	Unknown	13.2	Unknown	Unknown	Unknown	Only two possible arms visible; sharp	Scalloped edges	contaminated blank control 14  Could not rotate to definitively determine shape; visible only from side angle but arms rotate with polarizers; from sample two
Shamanka II	47-1	Likely oval	12.5	Unknown	Unknown	Unknown	Debatable cross but dark bands move with polarizers	Scalloped edges	Could not rotate to definitively determine shape; from sample two
Shamanka II	47-1	Round	5.5	Closed/Central	Absent	Absent	Quite strong birefringence, cross arms at right angles	Possible	Attached to small amount of calculus; from sample two
Shamanka II	60-2	Likely polyhedral	13.2	Closed/Central	Absent	Possible (obscured by cracking)	Strong birefringence, cross arms' sharp	Cracked in two while attempting to rotate	Could not rotate to definitively determine shape
Shamanka II	60-2	Round	8	Closed/Central	Absent	Present (Y- shaped)	Moderate birefringence and cross arm width, arms at right angles	None	Considered cautiously as a non-contaminant
Shamanka II	30-1	Roughly semi- circular	16.8	Closed/Unknown	Absent	Absent	Moderate birefringence and sharp cross arm width	None	Base of the grain comes to a point, where the hilum appears to be located; could not fully rotate; from sample one
Shamanka II	30-1	Likely round to oval	9.3	Closed/Central	Absent	Present (Y or X-shaped)	Weak, inconsistent birefringence though cross arms quite sharp	Inconsistent birefringence	Attached to calculus and could not rotate to definitively determine shape; from sample two
Shamanka II	30-1	Round	10	Closed/Central	Present	Absent	Inconsistent birefringence, wide cross arms	Inconsistent birefringence	From sample two
Shamanka II	46-1	Roundish/ oval	24	Closed/Central	Present	Absent	Moderate birefringence, wide cross arms	Cracking along edges and inconsistent birefringence	From sample one

Cemetery	Burial	Shape*	Size ( $\mu\text{m}$ )**	Hilum (open or close/central or acentric)	Lamellae (present or absent)	Fissures (present or absent/type)	Birefringence	Damage	Other/Notes***
Shamanka II	46-1	Unknown	11.5	Unknown	Absent	Unknown	Weak birefringence and very wide cross arms, barely discernable extinction cross	Scalloping, half of grain has been torn off	Possible slight depression in center; from sample one
Shamanka II	24	Roundish	16.5	Closed/Central	Absent	Absent	Strong birefringence and sharp cross arms	None	Grain a slightly darker color than others seen; attached to a small amount of material; small indentation at hilum
Shamanka II	76-1	Unknown	7	Closed/Central	Absent	Absent	Only two possible cross arms visible; sharp	None	Only a possible grain; could not be rotated to definitively determine shape
Shamanka II	66-1	Roundish/ polyhedral	13.4	Closed/Slightly acentric	Absent	Present (straight)	Moderate birefringence, fairly sharp cross arms	None	Fairly similar to some contaminants therefore should be regarded cautiously.
Shamanka II	26-3	Trapezoidal	8.2	Closed/Slightly acentric	Absent	Absent	Moderate birefringence, fairly sharp cross arms	None	Could not rotate to definitively determine shape; depression in center of grain
Shamanka II	26-3	Round	9.8	Closed/Central	Absent	Absent	Very weak birefringence, wide cross arms	Stellate cracking on surface	None
Shamanka II	67	Round/ lobed	8.8	Closed/Likely central	Absent	Present (deep fissures separate the grain into three lobes)	Moderate birefringence, hard to see cross arms due to lobes but rotated with polarizer	None	None
Shamanka II	67	Discoïd to convex- lenticular	11.9	Closed/Central	Present	Absent	Strong birefringence, cross arms fairly sharp	None	Protuberance at one end of grain
Ust'-Ida I	6-1-1	Convex- lenticular	33	Closed/Unknown	Present	Absent	Little to no birefringence	Considerable cracking	Lack of birefringence likely due to damage
Ust'-Ida I	6-1-1	Round	35.9	Closed/Central	Present	Absent	Very weak, cross arms wide	Cracking and inconsistent birefringence	Could not be rotated to definitively determine shape



Cemetery	Burial	Shape*	Size (µm)**	Hilum (open or close/central or acentric)	Lamellae (present or absent)	Fissures (present or absent/type)	Birefringence	Damage	Other/Notes***
Ust'-Ida I	38-1-1	Unknown	12.2	Unknown	Absent	Unknown	Only two possible arms visible; sharp	Only half of grain is present	Could not be rotated to definitively determine shape
Ust'-Ida I	45-1-1	Roundish-oval	22	Closed/Central	Absent	Absent	Moderate birefringence, moderate to wide cross arms	Cracking on edges	Circular outline in center of grain around hilum; considered only cautiously as a non-contaminant, as circular outline is seen on some grains in controls but size and shape were different
Ust'-Ida I	33-1-1	Discoid	17.4	Closed/Likely central	Absent	Absent	Cross arms seen on the edges of the grain but not in the middle	None	Small deep depression in center of grain
Ust'-Ida I	56-1	Oval to round	9.3	Closed/Acentric	Absent	Absent	Moderate birefringence and relatively sharp cross arms	None	One end of the grain comes to a point, hilum is located at opposite end from point
Ust'-Ida I	56-1	Unknown	<5	Unknown	Unknown	Unknown	Only two possible cross arms visible	Unknown	Possible grain; very small
Khuzhir-Nuge XIV	52	Roundish to oval	18.3	Closed/Central	Absent	Absent	Moderate birefringence, moderate to wide cross arms, no birefringence in center of grain	None	Large circular outline in center of grain with no birefringence, not a depression; from subsample two
Khuzhir-Nuge XIV	27-1	Likely polyhedral	18	Closed/Central	Absent	Present (shallow straight or Y shaped fissure)	Strong birefringence, sharp cross arms	Cracked in two while attempting to rotate	Could not rotate to definitively determine shape; from subsample one
Khuzhir-Nuge XIV	12	Semi-spherical with a double faceted base	16.8	Closed/Central	Absent	Absent	Strong birefringence, moderately sharp cross arms that widen toward the edge of the grain	None	Should be considered cautiously, a grain from a mandible swab associated with Ust 30-1-1 has a similar shape but has a fissure and birefringence is different; from sample one

Cemetery	Burial	Shape*	Size (µm)**	Hilum (open or close/central or acentric)	Lamellae (present or absent)	Fissures (present or absent/type)	Birefringence	Damage	Other/Notes***
Khuzhir-Nuge XIV	12	Convex-lenticular	24	Closed/Central	Absent	Absent	Weak, inconsistent birefringence and wide cross arms; H-shaped extinction cross	Inconsistent birefringence	From sample one
Khuzhir-Nuge XIV	35-1	Concave-lenticular	18.9	Closed/Central	Present	Absent	Weak birefringence and wide cross arms	Birefringence may indicate damage	From subsample two
Khuzhir-Nuge XIV	32	Roundish	4.5	Closed/Central	Absent	Absent	Moderate birefringence, sharp cross arms	None	Attached to demineralized calculus
Khuzhir-Nuge XIV	32	Roundish	4	Closed/Central	Absent	Absent	Moderate birefringence, sharp cross arms	None	Attached to demineralized calculus
Khuzhir-Nuge XIV	11	Oval	18.2	Closed/Central	Absent	Present (likely Y or X shaped)	Weak birefringence and cross arms	Appeared slightly gelatinized	Surface of grain is uneven and damaged; from sample one
Khuzhir-Nuge XIV	11	Discoid to slightly concave lenticular	15.8	Unknown	Absent	Absent	Weak, inconsistent birefringence and damaged cross	Birefringence indicates damage to center of grain	From sample one
Khuzhir-Nuge XIV	11	Roughly rectangular	31.5	Closed/Central	Absent	Absent	Extinction cross present on only one part of grain, cross arms at right angles, sharp	None	Very odd particle, unlike other grains seen; made up of two granular sections/pieces and only one side exhibits an extinction cross, other piece only exhibits very weak birefringence; possibly a complex grain; from sample one

\*Please refer to Appendix F for definitions to the various shapes; \*\*All measurements are of the longest dimension; \*\*\* subsamples refer to different aliquots drawn from the same processed sample while samples refer to different processed calculus deposits taken from the same individual or two parts of the same deposit processed on different occasions; Note: Eighteen starch grains considered to be contaminants not included

pressure to the coverslip. This suggests that there is a high likelihood these were embedded within the calculus before chemical dissociation.

Eighteen of the 49 starch grains exhibited signs of damage. While some of the damage may have been due to the acid treatment, diverse types of damage were observed (e.g., weakening birefringence, disruption of extinction cross, tearing, cracking, etc.; Figure 5.5). This suggests that not all of the damage was caused by acid. One even showed signs of gelatinization of the surface, often related to boiling and high temperatures (Figure 5.5.2a, b). This is discussed more in Section 5.4.

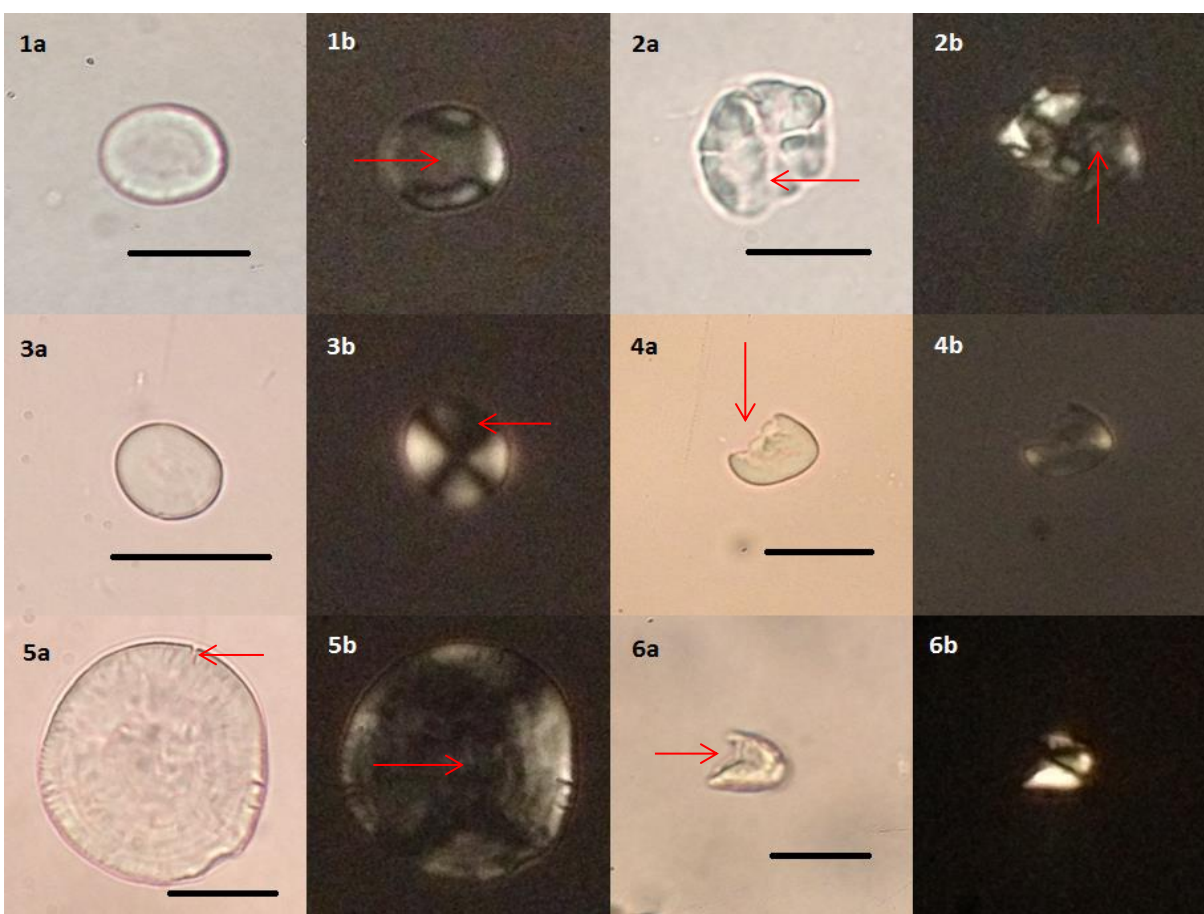


Figure 5.5 Examples of damaged starch grains from calculus associated with Cis-Baikal individuals, a=normal light, b=cross polarized light, red arrows indicate where damage is evident, scale=15  $\mu$ m: (1) Khuzhir-Nuge XIV Burial 11, sample one, disruption/blurring of extinction cross; (2) Khuzhir-Nuge XIV Burial 11, sample one, possible slight gelatinization; (3) Shamanka II Burial 30-1, sample two, disruption/blurring of extinction cross; (4) Shamanka II Burial 46-1, sample one, scalloping and half of grain torn off; (5) Ust'-Ida I Burial 6-1-1, disruption/blurring of extinction cross and cracking on edges; (6) Ust'-Ida I Burial 38-1-1, part of grain torn off.

Two other starch grains were of note. One starch grain from Khuzhir-Nuge XIV Burial 27-1 and one from Shamanka II Burial 60-2 cracked when the coverslip was tapped (see Appendix E). These were the only two cases where this happened, including within trial rounds involving modern/historic calculus samples. While this cracking may have been due to the acid treatment of the samples, many of the trial samples were processed with stronger acids and for longer periods of time with no starch grains behaving in this manner. It may be that these starch grains were more delicate when entering the acid, possibly due to their archaeological origin and advanced age. The acid may have further damaged the grains, resulting in them shattering upon contact.

### 5.2.3 Phytoliths

Very few phytoliths were found within the samples, and most were only tentatively identified as potential phytoliths. In total, 13 phytoliths and possible phytoliths were observed: seven from Lokomotiv, four from Khuzhir-Nuge XIV, one from Ust'-Ida I, and one from Shamanka II. Of the 13 particles identified, only three of these were confidently identified as phytoliths; two from Lokomotiv Burial L-9-1-1 including a trapezoidal and a roughly conical-flask shaped phytolith, and a rod shaped phytolith from Shamanka II Burial 30-1 (Figure 5.6; Table 5.3). The others exhibited only one or two characteristics considered indicative of phytoliths (see Chapter 3, Section 3.2.5). A summary of key characteristics of each of these phytoliths and possible phytoliths is provided in Table 5.3.

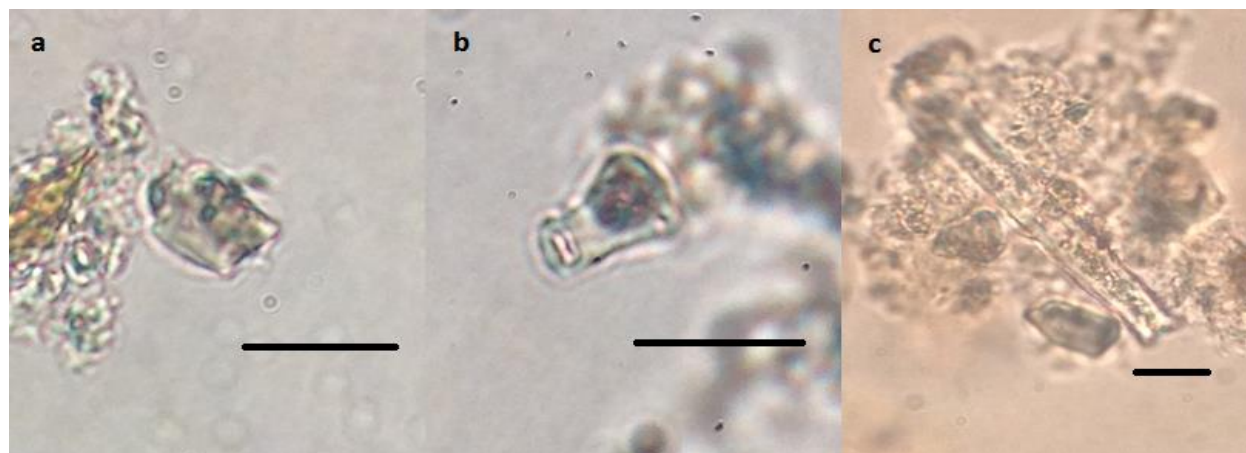


Figure 5.6 Microparticles identified as definite phytoliths recovered from calculus associated with Cis-Baikal individuals, scale=15 μm: (a,b) Lokomotiv Burial 9-1-1; (c) Shamanka II Burial 30-1, sample two.

Table 5.3 Summary of Key Characteristics of Identified Phytoliths found in Cis-Baikal Calculus Samples

Cemetery	Burial	Shape*	Size (µm)**	Colour	Other
Lokomotiv	R-11-1	Hook/trichome-like	13.3	Pinkish	Could not rotate to definitively determine shape; identification tentative
Lokomotiv	R-11-1	“Chef’s hat”	10	Opaque	Could not rotate to definitively determine shape; void/inclusion visible; identification tentative
Lokomotiv	R-11-1	Unknown	~11	Opaque	Could not rotate to definitively determine shape; embedded within calculus; void/inclusion visible; identification tentative
Lokomotiv	R-15-1	Trapezoidal	8.5	Opaque with pinkish hue	Could not rotate to definitively determine shape; embedded within calculus; identification tentative
Lokomotiv	L-9-1-1	Trapezoidal	12.1	Opaque/translucent with pinkish hue	Attached to a small amount of calculus
Lokomotiv	L-9-1-1	Conical-flask	12.9	Translucent	Attached to a small amount of calculus; inclusion/void at one end, other end may also have an inclusion/void but may just be something underneath transparent surface
Lokomotiv	L-9-1-1	Trapezoidal	10.7	Opaque to translucent	Could not rotate to definitively determine shape; attached to calculus; identification tentative
Shamanka II	30-1	Rod	~73	Opaque to translucent	Attached to calculus; from sample two
Ust'-Ida I	56-1	Pyramidal	25	Opaque	Could not rotate to definitively determine shape; attached to calculus; identification tentative
Khuzhir-Nuge XIV	52	Unknown	11.4	Opaque	Could not rotate to definitively determine shape; attached to calculus; identification tentative; from subsample one
Khuzhir-Nuge XIV	27-1	Conical	8.8	Translucent with pinkish hue	Could not rotate to definitively determine shape; attached to calculus; identification tentative; from subsample one
Khuzhir-Nuge XIV	27-1	“Chef’s hat”	10	Opaque to translucent	Could not rotate to definitively determine shape; attached to calculus; identification tentative; from subsample one
Khuzhir-Nuge XIV	27-1	Cylindrical	10.5	Opaque	Void visible; could not rotate to definitively determine shape; attached to calculus; identification tentative; from subsample two
Khuzhir-Nuge XIV	27-1	Unknown	15	Opaque to translucent	Void visible but not much structure distinguishable otherwise; could not rotate to definitely determine shape; identification tentative; from subsample two

\*Please refer to Appendix F for definitions to the various shapes; \*\*All measurements are of the longest dimension

No phytoliths were found in controls from the laboratory at the University of Saskatchewan. A few were found in control swabs taken from skeleton and container surfaces from the Irkutsk State University laboratory (Appendix D, Figure D-1) but were quite distinct from those in calculus samples. Phytolith contamination was therefore deemed minimal to absent in Cis-Baikal calculus samples. All observed phytoliths and possible phytoliths were likely incorporated into the calculus during the life of the individual

#### 5.2.4 Pollen and Spores

Pollen and spore identification was based on communications with Dr. Glenn Stuart and Dr. Elizabeth Robertson, both of the University of Saskatchewan. In total, five individuals had calculus deposits that contained tentatively identified pollen, while 28 individuals presented signs of spores or fungal related activities (Table 5.1). Pollen was identified based on ornamentation and, in some cases, the presence of apertures or arci (Glenn Stuart, personal communication 2014). An aperture is an opening on the surface of the grain associated with a normal exit of internal substances while arci are band-like local thickenings of the outer shell of the pollen grain extending from aperture to aperture (Erdtman 1952:459-460). All spores were considered as only tentative identifications, as most did not display the typical trilete, or Y-shaped, mark characteristic of spores; however, their other features were most consistent with this class of microparticles (Glenn Stuart, personal communication 2014). Fungal activity was predominately discerned based on the presence of hyphae associated with the varieties of spores (Elizabeth Robertson, personal communication 2014). Hyphae are microscopic tubular branching structures that make up most fungal bodies (Brooker et al. 2008:602).

Large clumps of pollen aggregates were seen in three individuals: Lokomotiv Burials R-11 and L-35-1-1, and Khuzhir-Nuge XIV Burial 34 (Figure 5.7). It was suggested that some of the particles within Lokomotiv L-35-1-1 resemble *Artemisia* (Glenn Stuart, personal communication 2014; Figure 5.7b, c), belonging to the family Asteraceae. Interpretations for the possible presence of this plant taxa are discussed below. Clumps from the other two Cis-Baikal samples were not identified to a particular plant. These pollen aggregates were considered to have been incorporated into calculus deposits in vivo based on direct association with calculus material (e.g., Figure 5.7c, d) and lack of comparatives observed in controls. The size and colour of these possible pollen grains were similar to small aggregates/spores of fungi seen in other

samples (see Figure 5.9 later in the section). It was often quite difficult to tell these apart, but the fungal aggregates often were closely associated with hyphae.

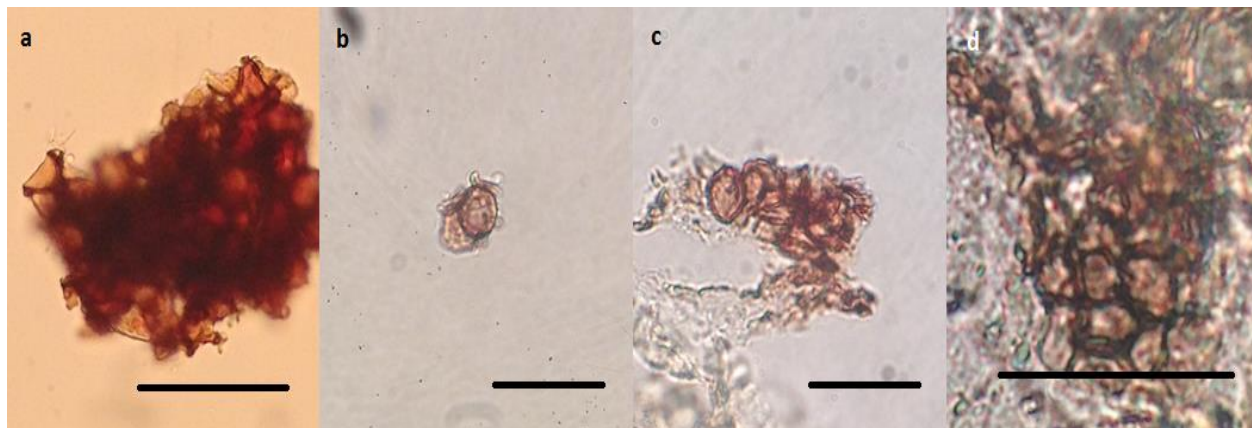


Figure 5.7 Pollen aggregates recovered from calculus associated with Cis-Baikal individuals, scale=25  $\mu$ m: (a) Lokomotiv Burial R-15-1; (b,c) Possible *Artemisia* pollen, Lokomotiv Burial L-35-1-1; (d) Khuzhir-Nuge XIV Burial 34.

Two other pollen grains observed as individual particles, rather than in clumps, were tricolporate (having three apertures). One was found in calculus from Ust'-Ida I Burial 38-1-1 and may represent a plant from the Primulaceae or Rhamnaceae family (Figure 5.8a; Glenn Stuart, personal communication 2014). The other, from Shamanka II Burial 30-1, sample two, was very poorly preserved but appeared to exhibit pores/apertures as well as arcs between the pores (Figure 5.8b). Stuart (personal communication 2014) suggests this could have originated from alder (*Alnus sp.*) or possibly elm (*Ulmus sp.*). Two other pollen grains were found in the same subsample from Shamanka II Burial 30-1. One was a fairly well preserved conifer pollen grain and the other was possibly a highly corroded *Equisetum* (field horsetail) pollen grain, though that is quite speculative (Glenn Stuart, personal communication 2014; Figure 5.8c, b). Firm attachment to demineralized calculus in some cases and lack of similarity to controls suggest incorporation during life.



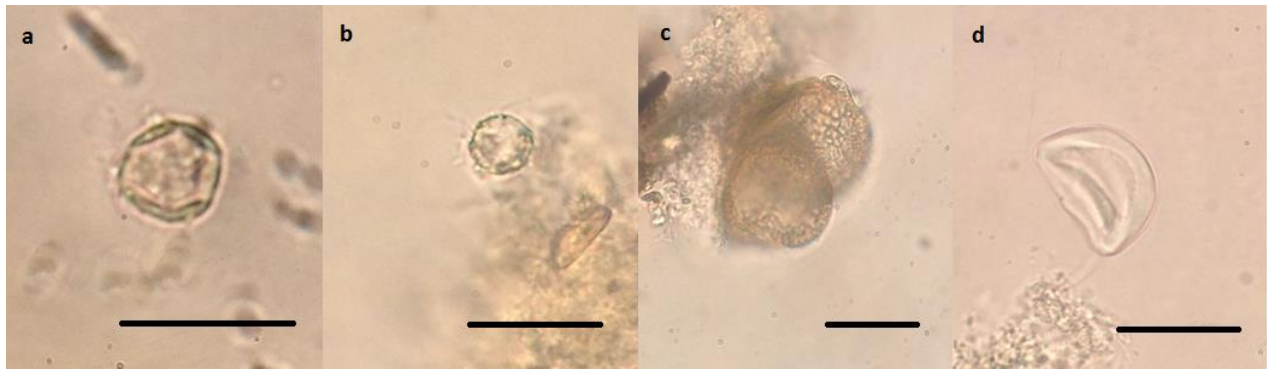


Figure 5.8 Pollen grains recovered from calculus associated with Cis-Baikal individuals, scale=25  $\mu$ m: (a) Tricolporate grain, possibly from the Primulaceae or Rhamnaceae family, from Ust'-Ida I Burial 38-1-1; (b) Possible degraded tricolporate grain, from Shamanka II Burial 30-1, sample two; (c) conifer pollen grain, from Shamanka II Burial 30-1, sample two; (d) Possible corroded pollen grain, from Shamanka II Burial 30-1, sample two.

A large number of spores were found within calculus subsamples, both as aggregates and individually. Most of these were considered to be related to extensive fungal activity. This included both small brown cell-like fungal spores/aggregates and larger brown to opaque spore particles (Figure 5.9). Both were often attached to fungal hyphae, ranging in colour from light brown to opaque, which was how they were distinguished from spores of other, unknown, origins. This fungal activity was present to varying extents in 27 subsamples analyzed. Only five subsamples exhibited spores possibly associated with other origins. This was based on a lack of similarity to spores directly associated with hyphae. Mold found in control swabs and particle traps were distinct from this fungal activity (see Appendix D, Figure D-8 for comparison), suggesting potential *in vivo* incorporation but, on the other hand, this activity may have originated from the burial, rather than the curatorial, environment. Much of the hyphae seemed to be completely incorporated and intertwined with the demineralized calculus deposits which suggested that fungal organisms had infiltrated the pores of the calculus.

Two other spores were interpreted as indicating postmortem fungal activity as well, but were distinct from fungal activity in all other subsamples. There was no evidence of associated hyphae for either particle. Both of these were found in calculus from Shamanka II Burial 109-1. The body of each was round and spicules/hairs extended out from the body, giving it a starburst shape (Figure 5.10a). The spicules/hairs were longer in one of the particles than the other. A similar particle was found in a bag control from Ust'-Ida I Burial 44-2 (Appendix D, Figure D-



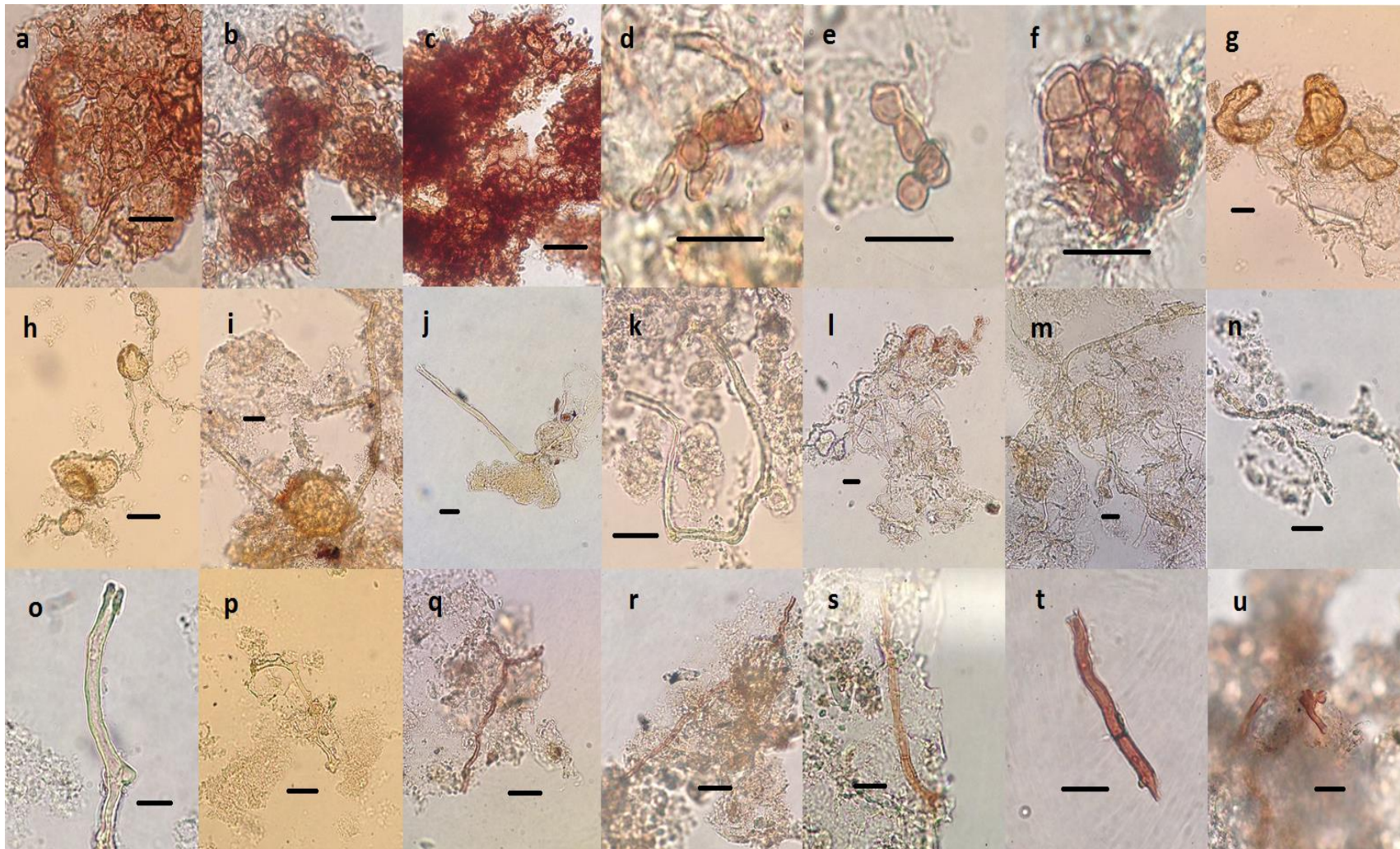


Figure 5.9 Probable postmortem fungal activity in Cis-Baikal calculus samples, scale=15  $\mu$ m: (a-f) small brown clumps of fungal spores; (g-i) large spores with attached hyphae; (j-p) opaque hyphae; (q-u) reddish brown hyphae.

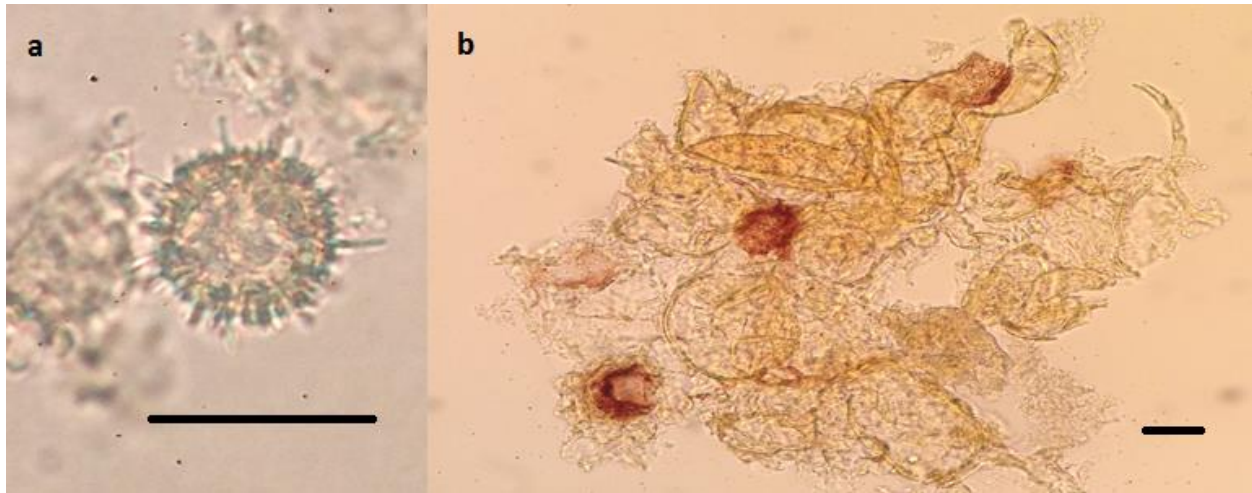


Figure 5.10 Spores recovered from calculus associated with Cis-Baikal individuals, scale=25  $\mu$ m: (a) Shamanka II Burial 109-1; (b) Shamanka II Burial 47-1, sample two.

8d), which indicated that this represented mold/fungus likely was a contaminant from the storage environment which had adhered to the surface of the calculus deposit.

A few other spores were identified, with a distinct appearance from the variety associated with fungal hyphae or the aforementioned starburst type. A reddish brown particle which was likely a spore was found embedded in calculus from Lokomotiv Burial L-28-1-1, sample one. Two highly degraded particles from Shamanka II Burial 46-1, sample two were likely spores, though no trilete marks were present (see Appendix E; Glenn Stuart, personal communication 2014). Four to five spores/spore fragments were also found still attached to demineralized calculus from Shamanka II Burial 47-1, sample two (Figure 5.10b). These were quite similar in appearance to *Lycopodium* spores but darker brown in colour. The firm attachment of these spores/spore fragments to calculus after processing of the sample may be indicative that these had been incorporated into the calculus during the individual's life.

### 5.2.5 Miscellaneous Particles

A few observed non-plant particles were of note. Charcoal fragments, identified based on Whitlock and Larsen (2001) and The McCrone Group (2005-2012), were found within calculus samples from three individuals, all from Khuzhir-Nuge XIV (Burials 35-2, 52, and 32; Table 5.1; Figure 5.11). These ranged in colour from blue-black to very dark brown, and various sizes and shapes were noted. Only one piece appeared to have a small amount of demineralized calculus



adhered directly to the particle (Figure 5.11e); the others were found loose on the mounted slides. A lot of solid dark debris was noted in control swabs although no charcoal fragments were noted specifically. Of the three burials, only Khuzhir-Nuge XIV Burial 52 exhibited signs of mortuary ritual use of fire. Still, small to large charcoal pieces were noted throughout the burial matrix for Khuzhir-Nuge XIV Burials 52 and 35-2; they were only noted in the upper portion of the sediment fill of Khuzhir-Nuge XIV Burial 32 (Weber et al. 2008). Wesolowski et al. (2010) presented a very similar case. They quantified charcoal presence within calculus deposits because they originally associated it with possible evidence of cooking over a fire. They also recognized that the deposits with the greatest charcoal concentrations all had hearths associated with the burial. In the end they concluded that these were likely contaminants from the burial environment that had adhered to the calculus deposits, staying on these surfaces even after cleaning due to their porous nature. It is quite likely that the situation is very similar here, although the possibility that it may represent fire use during the individual's life should not be completely discarded.

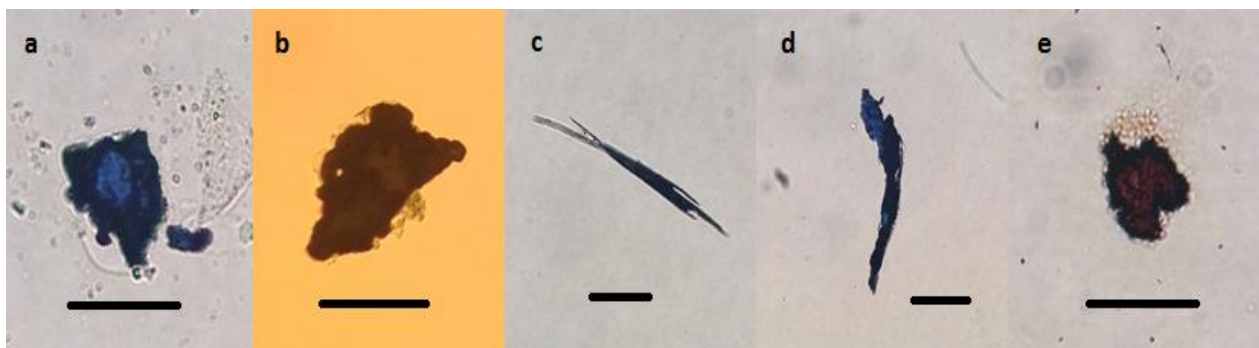


Figure 5.11 Likely charcoal fragments recovered from calculus associated with Cis-Baikal individuals, scale=25 microns: (a,b) Khuzhir-Nuge XIV 52; (c,d) Khuzhir-Nuge XIV 35-2; (e) Khuzhir-Nuge XIV 32.

Another particle, identified in the subsample from Shamanka II Burial 30-1, sample two was positively identified as a testate amoeba (Jacques Brochier, personal communication 2014; Microworld: World of Amoeboid Organisms 2015). Testate amoebae are ubiquitous protists that produce a shell called a test (Wall et al. 2010). In this case, only the test remained (Figure 5.12a). While it is possible that this was ingested through a water source and incorporated into calculus, a similar but highly degraded test was also found in a mandible control swab from Khuzhir-Nuge

XIV Burial 33 (Appendix D, Figure D-7b). This suggests that the test may instead have been a component of the burial or curatorial environment.

Two possible diatoms were noted, but identification was very tentative due to lack of comparatives. One, from Lokomotiv Burial R-15-1, was rod shaped, with scalloped edges, and reflected light slightly around its edges (Figure 5.12b). The other, from Shamanka II Burial 109-1, was partially embedded within calculus and therefore could not be seen in full but the body appeared to be ribbed or ornamented (Figure 5.12c). While not a dietary inclusion per se, diatoms and protists can provide useful information on water sources. Recall that Dudgeon and Tromp (2012) used diatoms in calculus to hypothesize an alternative water source for southern populations in Rapa Nui. While no such conclusions could be drawn here due to sample size (n=2) and lack of comparatives, the embedded nature of the second diatom indicated incorporation *in vivo* rather than postmortem, making it a viable avenue for future research.

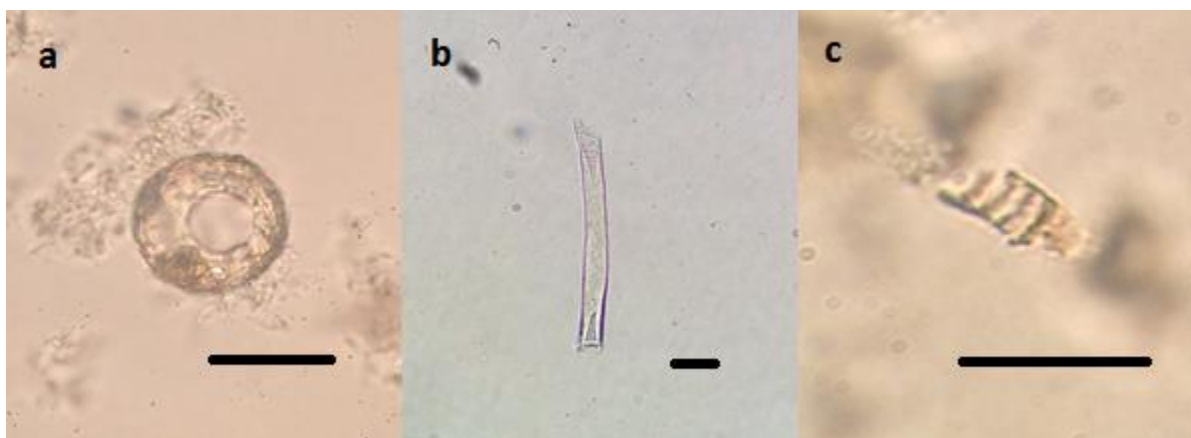


Figure 5.12 Miscellaneous particles recovered from calculus associated with Cis-Baikal individuals, scale=25  $\mu$ m: (a) Amoeba test, Shamanka II Burial 30-1, sample 1; (b) Possible diatom, Lokomotiv Burial R-15-1; (c) Possible diatom, Shamanka II Burial 109-1.

A variety of unknown particles were recorded during analysis. Some were opaque and likely mineral in origin while others appeared as possible tissue from unknown plant particle fragments (Figure 5.13). Many appeared to be directly associated with demineralized calculus, suggesting *in vivo* inclusion in the mouth. These are not discussed further within this study but they are reported in the event that future research discovers their source. Bacterial chains were observed in Khuzhir-Nuge XIV Burials 27-1 and 12. Both consisted of chains of rod-shaped bacilli bacterial cells. A study conducted by Moolya et al. (2010) showed that viable bacteria

were present within modern dental calculus samples, especially within internal channels and lacunae. Gram staining showed that this included both cocci and bacilli bacteria; therefore it is not unexpected to find them in the Cis-Baikal deposits. They will also not be discussed further.

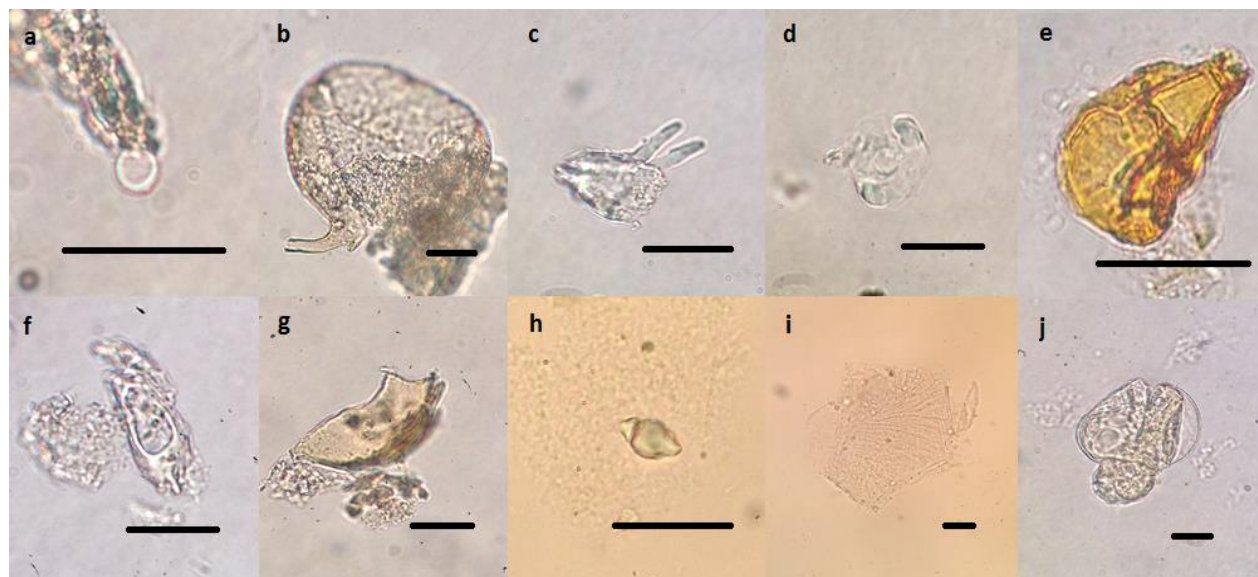


Figure 5.13 Unknown particles recovered from Cis-Baikal calculus samples, scale=25  $\mu$ m: (a) Unknown round particle from Lokomotiv Burial 35-1-1; (b) Unknown plant-like particle from Lokomotiv Burial L-20-2-1; (c,d) Unknown opaque particles from Lokomotiv Burial L-20-2-1; (e) Unknown plant-like particle from Khuzhir-Nuge XIV Burial 35-2; (f) Unknown opaque particle from Lokomotiv Burial L-9-1-1; (g) Possible plant particle fragment from Lokomotiv Burial L-9-1-1; (h) Unknown particle with triangular protrusions from Shamanka II Burial 46-1, sample one; (i) Unknown fibrous sheet-like material from Shamanka II Burial 30-1, sample one; (j) Unknown particle from Shamanka II Burial 46, sample two.

### 5.3 Summary of Results

Overall, there was a paucity of starch grains and phytoliths within dental calculus matrices of Cis-Baikal individuals. Counts for starch grains and phytoliths were generally between zero and four. For the most part, only a subsample of deposits was analyzed, so full counts for entire deposits would likely have been slightly higher. However, in addition to the single mounted subsample taken for each of the processed and analyzed calculus samples, second subsamples were mounted and scanned from seven individuals (Lokomotiv Burials R-14-1, L-38-2-1, L-15-1-1 and Khuzhir-Nuge XIV Burials 35-2, 52, 27-1 and 11). The results proved highly comparable to the first mounted and scanned subsamples for these individuals,

underlining the effectiveness of the scanning procedures while simultaneously demonstrating that the very time-consuming process of mounting and scanning entire samples from each individual provided limited additional information (Table 5.4; Appendix E). The first subsample from Lokomotiv Burial L-15-1-1 and Khuzhir-Nuge XIV Burial 35-1 was likely slightly underrepresentative of the whole sample, due to the observation of smaller amounts of calculus material on the slide compared to the second subsample, but they are included in Table 5.4 because it shows that even with slight underrepresentation, results in subsamples were still quite comparable. As such, the consistency of subsamples suggests that the true count of the entire sample processed can be extrapolated based on the relative proportion of the sample that was mounted for analysis. For example, since one-third to one-half of the sample was mounted, a starch grain count of two within the subsample would equate to roughly four to six starch grains total in the whole sample.

Table 5.4 Comparisons of Microscopic Results from Multiple Subsamples

Cemetery	Burial	Subsample 1	Subsample 2
Lokomotiv	R-14-1	No plant microparticles found	No starch or phytoliths; one small piece of possible plant tissue
Lokomotiv	L-38-2-1	No plant microparticles found	One starch grain (likely contaminant), no phytoliths
Lokomotiv	L-15-1-1	No plant microparticles found*	Two possible starch grains, no phytoliths
Khuzhir-Nuge XIV	52	One starch grain, no phytoliths, charcoal fragments	No starch grains, one possible phytolith, charcoal fragments
Khuzhir-Nuge XIV	27-1	One starch grain, two possible phytoliths	No starch grains, one to two possible phytoliths
Khuzhir-Nuge XIV	35-2	No starch grains or phytoliths, one unknown plant fragment, charcoal fragments	No plant microparticles, charcoal fragments
Khuzhir-Nuge XIV	35-1	One possible starch grain, no phytoliths*	Two starch grains, no phytoliths

\*May have been slightly less representative of entire sample (see Chapter 3, Section 3.2.3 and Appendix E)

Contamination was noted in controls from both the Irkutsk State University and the University of Saskatchewan laboratories and it resulted in the removal of some starch grains found in calculus samples from analysis as a precaution, to maintain rigorous analysis. Despite

this, a number of starch grains were considered to be quite distinct from contaminants and therefore were likely to have been incorporated into the calculus in vivo, making them reflective of these individuals' diets and/or activities. Some exhibit damage indicative of possible plant processing. No phytoliths were found within calculus samples that corresponded with contaminants, so all were retained in the analysis and offer archaeological insights regarding the diet and/or activities of those from whom these calculus samples were taken.

Other particles noted in the samples included pollen, spores/fungal activity, two possible diatoms, a testate amoeba, and charcoal fragments. Discussion with Dr. Stuart has tentatively identified a few pollen grains as originating from *Artesimia*, a plant from the Primulaceae or Rhamnaceae family, conifer, alder or elm, and possibly horsetail. Most spores appear to be associated with fungal activity, with many attached to hyphae, but whether this activity was antemortem, in the burial environment or in the post-excavation storage environment is debateable. Charcoal fragments were found in three individuals from Khuzhir-Nuge XIV and are likely of postmortem origin. While the pollen grains and diatoms were likely included within calculus deposits in vivo, the origins of the testate amoeba, as well as spores/fungal activity, are less certain.

## **5.4 Discussion and Interpretation**

Results were based on quantitative and physical observations of microparticles, and were compared to past research on microparticles within dental calculus as well as BHAP research on Cis-Baikal lifeways (e.g., Dudgeon and Tromp 2012; Henry and Piperno 2008; Li et al. 2010; Menéndez et al. 2009; Mickleburgh and Pagan-Jimenez 2012; Piperno and Dillehay 2008; Lieveise et al. 2007a; Waters-Rist et al. 2010; Weber et al. 2002; Weber and Bettinger 2010). This work was the basis for assessing the feasibility of using dental calculus for dietary and activity reconstruction in this population, thereby determining the potential research value of time-consuming ancillary studies such as collecting and processing samples from modern Cis-Baikal plant specimens to build the comparative starch and phytolith library needed for definitive microparticle identification.

Although quantities were quite low, starch grains and phytoliths were successfully observed and considered to have been in contact with the oral cavity during the life of some Cis-Baikal individuals. The low quantities of starch grains and phytoliths constitute an important outcome of this study. The contamination found in the controls from the Irkutsk State University

and University of Saskatchewan laboratories proved the extraction method was effective; yet little was found within the calculus samples and it was clearly not due to ineffective or otherwise problematic sample processing and preparation methods. Once possible contaminants were eliminated, remaining microparticles provided a number of insights into the lifeways of these Cis-Baikal populations.

First, the quantitative number of starch grains and phytoliths compares well with previous research indicating that plants played a less significant role in the diet of Cis-Baikal hunter-gatherers than did meat and fish (Weber et al. 2002; Katzenberg et al. 2010). The few starch grains recovered indicate the occasional consumption of starch, or starch incorporated into calculus through non-dietary mouth use or the environment. Plant microparticle counts reflect differences in the degree to which food procurement strategies emphasize plant foods, and the Cis-Baikal samples fit well within this. As a comparison, many past research projects have been very successful in the recovery of starch grains and phytoliths from calculus (Dudgeon and Tromp 2012; Henry and Piperno 2008; Henry et al. 2010; Li et al. 2010; Menéndez et al. 2009; Mickleburgh and Pagan-Jimenez 2012; Piperno and Dillehay 2008). Most of these research projects examined dental calculus samples from individuals belonging to agricultural societies, where dietary plant utilization was much more pronounced; therefore there were increased opportunities for plant particles to become trapped in calculus deposits.

Only a few studies have examined dental calculus from hunter-gatherer populations more directly comparable to the Cis-Baikal. A study on dental calculus from prehistoric sedentary fisher-gatherers associated with four Brazilian shell mounds provided an example of a population subsisting on a more mixed diet. Calculus samples from these individuals yielded starch grain counts that generally ranged between five and 20. Some of these grains represented cultigens, indicating some intensification in plant use. Counts for some Cis-Baikal individuals were not drastically different from the Brazilian fisher-gatherers, if you take into account that only a subsample was analyzed in this study. Yet, Cis-Baikal starch grain counts fell to the low end of the Brazilian range suggesting a decreased emphasis on plants.

Henry et al. (2010) examined seven dental calculus samples from three Neanderthal individuals, one from Shanidar Cave, Iraq, and two from Spy Cave in Belgium, and identified over 200 starch grains and 20 phytoliths. Microparticle frequencies were much higher in these Neanderthal individuals than in Cis-Baikal individuals, even when total counts were



extrapolated. This may be because these individuals were utilizing plants to a greater extent than Cis-Baikal individuals, or that the foods they were consuming were higher in starch frequencies or more readily incorporated into calculus. Mickleburgh and Pagan-Jimenez (2012) state that consumption of a plant does not guarantee that its starch grains will be preserved in calculus and in some cases starch grains cannot be identified, since not all plants produce diagnostic starch grains. For example, Weber et al. (2002) state that pine nuts would have been seasonally available to Cis-Baikal hunter-gatherers and are known to have been exploited by indigenous Siberians based on ethnographic accounts. For the purposes of this study, modern pine nuts, obtained in Siberia, were crushed and analyzed microscopically to compare with starch grains found in calculus samples. Pine nut starch grains were found to be very small and featureless, with faint birefringence, making them quite difficult to observe (Figure 5.14). They were also quite sparse when compared to samples prepared from foods such as potato or maize, so they likely would not have been incorporated as readily into dental calculus. The small size and low observability of the pine nut grains likely impair their preservation or recoverability. Therefore, it is quite likely that these would be underrepresented archaeologically. No starch grains associated with calculus were confidently identified as pine nuts, but some of the small starch grains observed in calculus displayed some resemblance (e.g., Khuzhir-Nuge XIV Burial 32, see Appendix E).

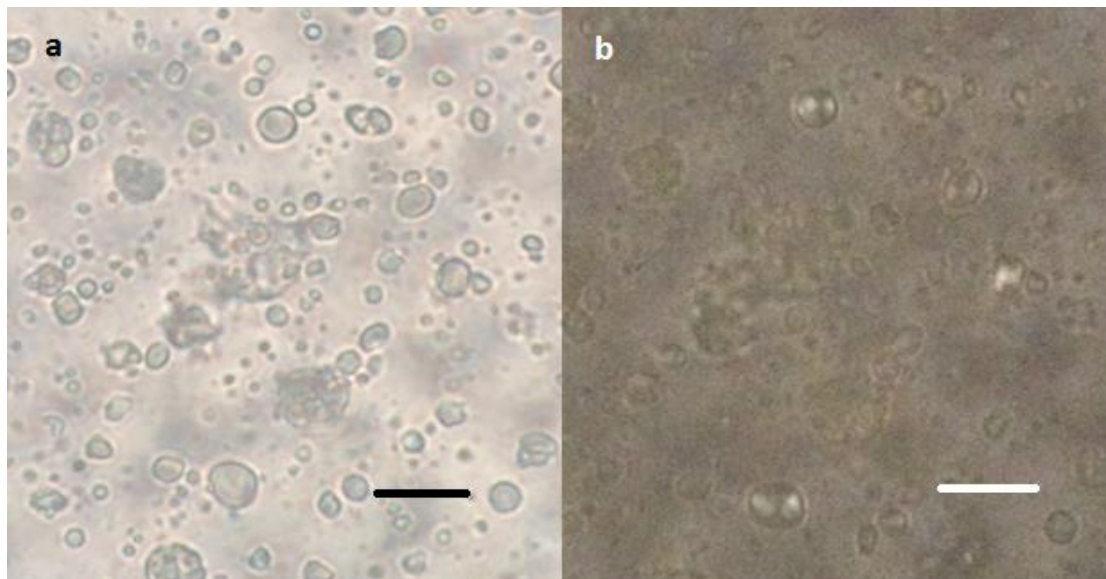


Figure 5.14 Comparative modern pine nut starch grains, scale=10  $\mu$ m: (a) Starch grains under normal light; (b) Starch grains under cross polarized light.

Second, the data suggest that, while likely not present in large quantities, plants were available as an alternate nutrient source. This conclusion is strengthened based on observations of damage to a number of starch grains observed. While some of the damage was likely due to the acid treatment used during sample processing, diverse types of damage were observed, suggesting that not all of the damage was caused by acid. Other explanations for the damage may include poor preservation, environmental damage (either ante- or postmortem), trauma from chewing, or plant processing. Past research has looked at many different plant processing techniques such as boiling, baking, and milling/grinding (e.g., Beck and Torrence 2006; Hardy et al. 2012; Henry and Piperno 2008; Henry et al. 2009; Yang and Perry 2013). Birefringence damage, cracking and tearing are known to occur with such techniques. Notably, a starch grain associated with Khuzhir-Nuge XIV Burial 11 exhibited signs of slight gelatinization on its surface. Gelatinization occurs when a starch grain is heated in the presence of sufficient moisture; the grain absorbs water, causing it to swell, which irreversibly disrupts the crystalline structure and results in a paste-like texture (Copeland et al. 2009). Cooking and boiling foodstuffs are known to cause this, creating desirably softer, more edible food textures. Pottery has been found associated with each cemetery population, although to varying extents (Weber 1995; Weber et al. 2002; Weber et al. 2008; Bazaliiskii 2003; Bazaliiskii and Savelyev 2003), providing further evidence that cooking/processing of food stuffs took place. This should be considered cautiously however, as Collins and Copeland (2011) note that gelatinization is also a natural step in diagenesis over time. Edge cracking, noted on a number of starch grains recovered from Cis-Baikal dental calculus samples, is suggested to be a result of dry heating/parching (Hardy et al. 2012), another cooking method. Hardy et al. (2012) hypothesize that dry heating may actually harden the grain casing, allowing for better preservation; this may explain the representation of these grains within the samples. Taken together, the damaged grains may indicate that plant processing did take place in the Cis-Baikal and that plant microparticles were just not incorporated into dental calculus readily. While it is beyond the scope of this research project, if future research proves this starch grain damage to be a cultural consequence, it may provide some key insights into plant processing among these populations.

Third, the microparticle analysis also sheds important light on mouth use patterns in the Cis-Baikal. The lack of phytoliths within most samples was quite surprising considering the large number of activity-induced dental modifications previously noted on teeth (Waters-Rist et al.

2010). Waters-Rist et al. (2010) concluded that striations associated with occlusal grooves support the interpretation that plant fibers or sinew were likely manipulated with the teeth, possibly related to basket weaving, net manufacturing, etc. If structural components of plants were being run through the teeth, likely some of the phytoliths common in such structural tissues would have become incorporated into dental calculus, as was the case in the study conducted by Blatt et al. (2010). Sample one from Shamanka II Burial 46-1 was taken for the express purpose of exploring this hypothesis. The right maxillary second premolar had a large rounded enamel defect on its mesial surface consistent with non-dietary mouth use and also had a substantial amount of calculus on the buccal surface of the crown (Figure 5.15). However, no phytoliths were recovered. For that reason, the results do not provide additional evidence for the utilization of plant fibers in non-dietary mouth use activities. It is therefore more likely that sinew or other material was manipulated within the oral cavity causing the grooving noted by Waters-Rist et al. (2010). All the same, plant fibers should not be completely disregarded since these populations may have utilized parts or species of plants that did not contain large numbers of phytoliths or phytoliths may not have been readily incorporated into calculus because they were not liberated by the processing in the oral cavity. Some plants/plant parts do not contain phytoliths at all (Piperno 2006:6).



Figure 5.15 Tooth 15 from Shamanka II Burial 46-1, showing extensive dental modification to the mesial surface resulting in a large rounded defect and pulp exposure.

Lastly, pollen grains, diatoms and possibly some spore fragments are likely not related directly to the diets of Cis-Baikal hunter-gatherers but offer potential information regarding palaeoenvironment, activity patterns, and non-dietary mouth use. It is possible that pollen grains, diatoms, and spores were environmental contaminants from the burial environment but none were found to match particles observed in control swabs (Appendix D) and some were found firmly embedded within calculus during microscopic observation. Taken together, there is good evidence that these were incorporated into calculus *in vivo*.

The pollen grains recovered seem to represent mostly non-food plants that may have originated from the middle Holocene Cis-Baikal environment. These may have been exploited by Cis-Baikal populations for medicinal or utilitarian purposes (e.g., basket weaving) or may have simply become incorporated in dental calculus through grit or water sources or even just breathing through the mouth. The pollen grains possibly identified as *Artemisia* may have had a medicinal use. *Artemisia* is a genus that includes species such as mugroot, wormwood, and sagebrush, which contain essential oils and have strong aromas (Tannas 2004). Most species are quite bitter, but a few are used as herbs and medicinal remedies (Elfawal et al. 2012; Sadiq et al. 2014; Obolskiy et al. 2011). *Artemisia* is known to exist in eastern Siberia, and actually gets its specific name from the harsh climate of eastern Siberia (Tannas 2004). The genus has been noted in palaeoenvironmental studies associated with the BHAP (e. g., Bezrukova et al. 2010, 2013). Sagebrush species have been used extensively by North American First Nations people for flavoring, incense, and as a deodorizer. Seeds were also pounded into meal (Tannas 2004). The presence of the conifer pollen grain, on the other hand, may indicate a close interaction with this resource, possibly related to the harvesting of pine nuts, the edible seeds of the Siberian pine. More work is needed, mainly the development of a comprehensive comparative collection of plants available in the Cis-Baikal, to support or refute this identification.

Diatoms are usually found within aquatic environments; hence their presence within calculus has the potential to provide information on water sources. Sample size was extremely small ( $n=2$ ) though, and no comparative samples of diatoms were available from water sources in the Cis-Baikal area for this study, so no further conclusions can be drawn at this time. Potential diatom recovery provides an interesting avenue to future research nevertheless (see Chapter 6).

Other particles, including spores, charcoal, and the amoeba test, have a more unclear origin. With regards to spores, on the one hand, prior BHAP research noted that mushrooms were likely consumed by middle Holocene hunter-gatherers (Weber et al. 2002). The small brown cell-like spores noted in calculus samples were moderately similar to mushroom spores observed on an online microparticle database (The McCrone Group 2005-2012), though not an exact match. A recent study by Afonso-Vargas et al. (2015) identified parasitic corn fungal spores within dental calculus and, after intensive investigation, they concluded the spores had not come from the burial environment. This increased knowledge of corn consumption within the population of interest. The Cis-Baikal presents a similar case and it is possible that the small brown cell-like spores found in the Cis-Baikal samples could also have an ancient origin. Yet many of these brown cell-like spores were noted in direct association with hyphae that is more likely to represent postmortem fungal growth in calculus pores in the burial environment.

The extensive proliferation of hyphae in some samples somewhat dissuades the researcher from concluding that these fungal particles were incorporated into calculus during the life of the individual. Fungal hyphae proliferate in environments with adequate moisture and oxygen (Miles and Chang 1997) and room to grow. The burial environment would likely be much more conducive to this growth than from within a calcified matrix. The fungi and other particles would have then infiltrated the slightly porous exterior of the dental calculus deposit, resulting in the growth seen on slides. Yet, it is also possible that the hyphae entered the oral cavity before mineralization, from fungal food sources, rotten food, parasitic fungi, etc. This may have even been hazardous to the health of the individuals. Either scenario could explain the extensive intertwining of calculus and hyphae. Activity such as this was not seen in the controls. This is not saying that mold was not observed within the controls; however, it was strongly dissimilar in appearance (see Appendix D, Figure D-8 and Figure 5.9 for comparison) suggesting a separate origin. At this point, a substantive claim cannot be made one way or the other but this would be a useful subject for future research, as it has the potential to provide new information on both diet and health (see Chapter 6). Some other spores, not associated with fungal hyphae, were noted and appeared to be embedded within demineralized calculus, suggesting a possible ancient origin, perhaps related to diet and/or the palaeoenvironment.

The testate amoeba shell within the sample from Shamanka II Burial 30-1, sample two could have been ingested with fresh water but, due to the presence of another test found within a

control swab (Appendix D, Figure D-7b), it is considered here to be a contaminant from the burial environment. Testate amoebae are most often found in/near fresh water, as well as mossy soils (Encyclopedia Britannica 2015). While it is possible that this was incorporated through water sources during the life of the individual, the degraded test shell found in a control swab suggests otherwise. This may instead be a contaminant introduced during curation (e.g., cleaning specimens with non-distilled water) or from the burial environment, where both Shamanka II and Khuzhir-Nuge XIV are likely both suitable habitats for testate amoeba, as they both are located near fresh water sources. Similarly, charcoal may provide evidence of use of fire during the lives of the sampled individuals, but more likely was introduced to the calculus deposits from the burial environment, through the mortuary ritual use of fire, similar to the case documented by Wesolowski et al (2010).

Based on these results, it is feasible to obtain archaeological plant microparticles from dental calculus from Cis-Baikal individuals, with the potential to provide new information on diet and mouth use patterns in the region. However, the history of these collections generated some obstacles, which may deter immediate pursuit of this approach, especially as important ancillary activities, like gathering appropriate comparative plant microparticle collections, will be time-consuming. Notably, high levels of contamination in the Irkutsk State University laboratory indicated that past and present handling and storage practices have introduced plant microparticles to the calculus samples, decreasing the confidence with which ancient starch grains and other microparticles can be identified. Careful monitoring of contaminants, as outlined above, offered some solutions but was time intensive and did not preclude that all modern starch grains contaminants were identified and eliminated. If access to a positive pressure fumehood, or similar contaminant-free facility could be granted, many of the problems seen at the sample processing and collection stages could be reduced, although the history of potential contamination during curation cannot be resolved. However, if new burials are exhumed with caution, post-excavation/curation contaminants could be minimized. This would involve the use of sterile powder-free gloves during excavation and curation, and constant separation from food/food containers. Samples should be isolated from the external environment as soon as possible to decrease opportunities for contamination so any plant particles found could be more confidently associated with the life of the individual. Finally, if a comparative collection

of Siberian flora could be compiled, even the small amounts of data available in dental calculus may provide significant dietary, activity, and/or environmental insights.

## 5.5 Chapter Summary

To conclude, plant particles were present within dental calculus samples from the Cis-Baikal, albeit in very low quantities. Starch grains, phytoliths, pollen grains, and diatoms were positively identified and likely became embedded within the calculus of middle Holocene hunter-gatherers in the Cis-Baikal over their life spans. Low starch counts suggest that starchy foods played a less prominent role in the diet than meat, which supports past research (e.g., Weber et al. 2002; Katzenberg et al. 2010); however starch grains may be underrepresented within the calculus due to a lack of incorporation or poor preservation. Regardless, damage to starch grains indicate that plants were likely important enough to have been processed for consumption by the middle Holocene occupants of the region. Starch grains may have also entered the calculus through mouth use or environmental agents as well. These populations used their mouth extensively as tools (Waters-Rist et al. 2010) but it does not appear that plant tissues containing phytoliths (e.g., stalks, leaves) were a part of this process or were processed in a way that liberated phytoliths while the plant tissues were in the oral cavity. Many plants do not produce phytoliths at all (Piperno 2006:6) and would therefore not be represented in deposits. The presence of pollen, such as *Artemisia*, suggests possible medicinal or utilitarian plant-use, although pollen may have entered the oral cavity unintentionally through grit, water sources, or breathing through the mouth. Diatoms may provide information about water sources. More research needs to be conducted to make any conclusions on this. Taken together, microscopic analysis of dental calculus proved to have valuable potential to provide new information about Cis-Baikal lifeways, but also outlined some of the limitations of conducting this research on previously excavated material, due to the risk of contamination. Work needs to be done to create better and/or new contamination preventative measures before the full potential of this method can be achieved.

## **Chapter 6**

### **Summary and Conclusions**

#### **6.1 Macroscopic Analysis of Dental Calculus Severity**

The first objective of this research was to examine the severity of dental calculus from EN and LN-EBA groups in the Cis-Baikal in order to define similarities and differences within and among the populations. This was done by developing calculus indices from standard tooth surface scores. Severity patterns were discerned through non-parametric Mann Whitney U and Kruskal-Wallis testing and used to investigate whether or not dental calculus was an adequate proxy for cultural factors, such as diet and mouth use activities, in the Cis-Baikal. Based on stable isotope ratios (Katzenberg and Weber 1999; Katzenberg et al. 2009, 2010, 2012; Weber et al. 1998, 2011; Weber and Goriunova 2013) and archaeological data (Losey et al. 2008, Nomokonova et al. 2009; Weber et al. 1993, 1998), previous studies proposed that both EN and LN-EBA populations subsisted on a high protein/low carbohydrate diet, specifically focused on local game and fish. Although the populations on either side of the middle Neolithic biocultural hiatus (7,000/6,800 cal BP to 6,000/5,800 cal B.P.) exploited varying quantities of aquatic and terrestrial resources, these data indicated that protein ratios were likely similar throughout the middle Holocene (Lieverse et al. 2007a) and thus, dental calculus severity should have been quite similar if diet, specifically the proportion of protein to carbohydrates, was, indeed, the dominant factor affecting calculus formation.

There were a number of general calculus severity trends highlighted during statistical analysis. First, post-hoc testing tended to group lakeshore populations (Shamanka II and Khuzhir-Nuge XIV) together and riverine populations (Lokomotiv and Ust'-Ida I) together, not EN populations (Shamanka II and Lokomotiv) and LN-EBA populations (Ust'-Ida I and Khuzhir-NugeXIV). This pattern was noted for both the juvenile and adult male groups. This likely indicates that microregional settings were having a greater observable effect on dental calculus formation than were genetic/cultural affiliation or the overall proportion of dietary protein to carbohydrates. Microregional effects may be related to the environment (e.g., distinct water mineral contents) or resource availability/allocation (e.g., distinct diet resulting from microregionally adapted hunting and mobility patterns). Past BHAP research has also noted



microregional variability in certain activity patterns, oral pathological lesions and accumulations, and stable isotope ratios (Lieverse et al. 2007a, 2013, 2015; Waters-Rist 2010; Haverkort et al. 2010).

Second, significant differences in calculus indices between males and females from Shamanka II, but not from the other three cemeteries, suggested gendered social differentiation at Shamanka II, possibly related to unequal access to certain resources. Alternatively, this pattern might reflect greater microregional influences on Shamanka II male logistical hunting parties compared to Shamanka II females and to both males and females from other cemetery groups who spent the majority of their time at central residential locations or foraging across microenvironments that were microenvironmentally similar to those home bases. Also, fewer differences in severity scores were detected when adult females from each population were compared to one another than when males were compared. Kitoi populations generally had low residential mobility and restricted annual ranges (Weber et al. 2002; Weber and Bettinger 2010) but stable carbon and nitrogen analysis has also shown that signatures were more similar when Shamanka II and Lokomotiv were compared to each other than when compared to other EN cemeteries (Katzenberg et al. 2010; Weber and Bettinger 2010). This may have been due to similar adaptive strategies, group interactions, and/or overlap in fisheries among these two populations (Weber and Bettinger 2010); therefore, the lack of differences between female groups is not surprising. But, since Kitoi males were likely engaged in logistical foraging, they would have spent considerable time in areas beyond their home bases (Weber et al. 2002; Weber and Bettinger 2010). Shamanka II males may have encountered environmental/resource conditions much different than both Lokomotiv Kitoi populations as a whole and females within their own population. This further suggests that calculus severity was impacted by microregional effects linked to logistical foraging. If so, whatever the nature of these effects, they were only evident in the calculus data, rather than generating detectable variations in the isotope data for Kitoi males in the Shamanka II population.

Third, dental calculus severity increased in adults when compared to juveniles but an increase was often not seen when comparing older adults to younger adults. Severe dental wear was also noted on adults of increasing age (Lieverse et al. 2007a). This correlation suggests that dental calculus was removed from the tooth surfaces due to dental wear over time. Patterns for older adults were therefore likely skewed.

Fourth, significantly higher calculus severity was observed among adult males from Shamanka II when compared to those from the other cemetery populations only for deposits located in the anterior quadrants of the mouth. Mouth use activities that encouraged rather than removed calculus may have caused this disproportionate quantity of calculus to form among the incisors and canines. Negative correlation between severity patterns and occlusal grooving on surfaces considered to be associated with fiber or sinew utilization (Waters-Rist et al. 2011) suggest that this type of mouth use did not significantly increase calculus accumulations and in fact possibly decreased them. At this time it is unknown what other mouth use activities were employed, but smoking or chewing non-dietary plants, such as betel leaf, has been noted in past research to increase calculus deposits (Anerud et al. 1991).

Fifth, very large outliers were present in the data but were not a product of analytical error. It is irresponsible to remove outliers without just cause, and in this case their presence suggests that another factor was important in determining severity of dental calculus in the Cis-Baikal: individual variation. Instead these likely represented heavy calculus formers within the populations that were not averaged out due to small sample sizes. Lastly, at least some post-excavation removal of calculus took place on Lokomotiv and Ust'-Ida I individuals but overall general principals of calculus accumulation were upheld indicating that while the data needs to be regarded cautiously, they still provide useful information on severity in Cis-Baikal populations.

Overall, dental calculus severity indices indicate that diet alone does not accurately explain the patterns in the data. Instead, this research highlights the importance of seeking out all possible explanatory variables. A complex multifactorial model involving microregional differences in resources/environment, foraging patterns, individual variation, and dental wear patterns provides at least a partial explanation for the data trends observed.

## **6.2 Microscopic Analysis of Microparticles in Dental Calculus**

The second objective of this project was to directly observe microparticles that were incorporated into dental calculus through diet, mouth use, or other activities in vivo and to make a recommendation on the feasibility of this type of research to provide useful information on Cis-Baikal lifeways for future projects. This was done by dissociating calculus samples in HCl and analyzing the residue through an optic light microscope to detect any microparticles liberated by this procedure. Potential contaminants from the burial and laboratory environments were

carefully monitored and controlled by the inclusion of a stringent contamination protocol. Contamination was found to be quite high in the Irkutsk State University laboratory. In particular, swabs of curational containers and skeletal elements associated with the individual sampled often yielded large numbers of starch grains. Contamination was controlled and minimized at the University of Saskatchewan laboratory by strict protocols; however, small amounts of contamination were still noted in some instances due to the age and multifunctionality of the Archaeology and Anthropology Building. All starch grains, phytoliths, and other particles associated with Cis-Baikal calculus material were compared with contaminants and a portion of particles identified were found to match these controls. Particles that were found to match contaminants were excluded from further examination in order to minimize any risk of mixing modern examples with ancient ones for the purposes of this analysis.

Even using this rigorous process of elimination, a wide range of particles thought to be of ancient origin was found, albeit in low concentrations. Starch grains, phytoliths, pollen, spores, fungal hyphae, diatoms, charcoal fragments, and other unknown possible plant debris were identified. The low numbers of ancient plant microparticles are consistent with a diet based predominately on meat sources, but they also provide some of the first direct evidence of plant use in the Cis-Baikal. Starch grains, phytoliths, pollen, and diatoms were found to have been incorporated into calculus *in vivo*. Some starch grains exhibited damage consistent with plant processing techniques such as cooking and grinding, suggesting that, while plants only seem to have been utilized in low quantities, they were an important alternate nutrient source in the Cis-Baikal. Damage could also be related to natural starch degradation over time, however. Whether processed or not, the identification of starch grains that were clearly incorporated into calculus provide strong evidence for their use as food. Phytoliths, pollen grains, and diatoms likely represent non-food plant exploitation, incorporated into dental calculus through mouth use activities, medicinal practice, etc. On the other hand, they may represent aspects of the palaeoenvironment, incorporated through ingested grit, drinking water, or breathing through the mouth. The origins of fungal spores and hyphae, charcoal fragments, and an amoeba test are less certain and instead are more likely contaminants from the burial or curatorial environment. The successful retrieval of plant microparticles from ancient calculus demonstrates that this research has the potential to be very useful in future studies, although it also highlights the limitations of

working with previously excavated materials, particularly the risks of postmortem contamination.

### **6.3 The Utility of Dental Calculus – Dietary and Mouth Use Reconstruction and Beyond**

Together, the components of this research project highlight the usefulness of dental calculus in providing information on diet, as well as many other aspects of hunter-gatherer lifeways. The two components of this study are very different forms of analysis but they complement each other in many ways. Dental calculus severity looks at the end product of a multitude of variables acting on calculus formation processes throughout the life of the individual, while microscopic analysis provides a direct look at particles introduced into the oral cavity. The results provide strong evidence supporting past research on diet and microregional differences among Cis-Baikal populations (Katzenberg and Weber 1999; Katzenberg et al. 2010; Weber et al. 2002; Weber and Bettinger 2010; Lieverse et al. 2007a, 2013, 2015; Waters-Rist 2010; Haverkort et al. 2010), but also add important new information on Cis-Baikal lifeways.

Both methods reinforce the understanding that Cis-Baikal hunter-gatherers emphasized meat/fish resources in their diets, with plant foods playing a secondary role. Also, of utmost importance, this project moves beyond reiterating past research by providing significant new insights into the dietary/cultural dynamics of these populations. The microscopic observation of starch grains with diverse damage suggests that starchy foods may have been processed and consumed in low quantities, perhaps seasonally. In addition, severity trends, especially the high calculus index values among Shamanka II adult males relative to females, suggest the possibility of varying dietary resource allocation among hunting parties versus the home base populations.

The calculus data also offer explanatory power far beyond dietary observations. They suggest that genetic/cultural affinity had little effect on calculus formation and instead that the microregion in which a population resided had a much greater role in determining severity. Plant microparticles found within calculus complement this, as pollen and diatoms may represent aspects of the palaeoenvironment associated with home ranges. The latter may specifically offer the potential to link populations to specific water sources. Differing mineral content water sources may also be reflected in varying levels of calculus formation.

This study provided significant new information on mouth use in the Cis-Baikal. Shamanka II males were seen to have higher levels of calculus accumulation in anterior quadrants than other cemetery populations. While distinctions in Shamanka II males may be

related to aspects of logistical foraging, discussed above, these results also indicate that Shamanka II males may have engaged in a mouth use activity that specifically affected plaque or calculus in anterior teeth. Waters-Rist et al. (2010) noted that occlusal grooving, thought to indicate possible fiber and/or sinew utilization, was predominant in anterior teeth, but the frequency was much higher in females than males and riverine populations than lakeshore populations. Moreover, severity trends were generally negatively correlated with dental modifications of this nature. While fiber/sinew manipulation with the teeth may have been performed, it does not seem to have increased calculus severity in any significant way and may in fact have decreased it to some extent. The lack of phytoliths recovered during microscopic analysis furthermore suggests that material manipulated in the mouth may not have been plant based, or at least not have been from a plant, or a part of a plant, containing phytoliths. Together the dental modification data (Waters-Rist et al. 2010) and dental calculus data indicate that the oral cavity may have been used as a tool, or for other non-dietary uses, in more than one way. Calculus data indicate that, if any of these populations engaged in a mouth use activity that increased severity rates, it would have been an activity affecting Shamanka II males and involving manipulation with anterior quadrants of the mouth. It is unknown what these other mouth use activities could have been, but activities such as ceremonial smoking or chewing of unknown plant materials could be explored.

#### **6.4 Future Directions**

The results presented here not only support previous research and contribute new information regarding the Cis-Baikal's middle Holocene, but they also highlight many different possibilities for new research. This work also clarifies limitations associated with both datasets, such as sample size, postmortem removal of calculus, and contamination. These limitations will need to be carefully considered and/or overcome in future research.

The principal recommendation with regards to expanding on this study's results on severity of calculus among Cis-Baikal populations is to increase sample size by adding more cemeteries. Of importance would be the inclusion of newly excavated cemeteries. With a full understanding of the usefulness of ancient dental calculus, measures could be taken to ensure the preservation of calculus on the tooth surface, thereby avoiding the biases introduced by post-excavation cleaning like that seen in the Lokomotiv and Ust'-Ida I specimens. Statistical analyses presented here suggest that lakeshore populations share greater similarities in calculus

severity than do riverine populations, but tooth fragmentation/unobservability rates were high at Khuzhir-Nuge XIV, resulting in generally lower representation of tooth surfaces and low sample sizes overall. The addition of information from other lakeshore and riverine sites would be very valuable to exploring microregional differences in calculus severity.

As stated previously, access to seal resources may have had an effect on calculus formation in populations situated on, or with regular access to, the shores of Lake Baikal. Unfortunately, pinpointing the effect(s) of lipid and other molecular components of seal meat that may facilitate calculus formation would depend upon large-scale clinical trials over extended periods of time, and would generally be outside of the scope of bioarchaeological research. However, the Cis-Baikal samples could be compared to other hunter-gatherer populations known to exploit seal and other aquatic mammals such as whale and walrus. Correlations could strengthen the hypothesis that seal meat may have had an effect on calculus formation.

Also, while this study demonstrated the strengths of using calculus indices to study population trends, it also highlighted some issues when dental wear is extensive and post-excavation biases are evident. The incorporation of both presence/absence data and severity indices may be the most effective strategy going forward when previously excavated remains are used. Severity indices would only be calculated for individuals with deposits present. Inclusion of the presence/absence data would allow for the elimination of the zero scores within the severity data set while not actually eliminating the data provided by individuals or quadrants lacking calculus. The presence/absence data and severity data could be compared to attempt to pinpoint where inconsistencies may exist. For example, high absence rates should mean that little calculus formation occurred in the population and therefore severity should be low. But if these data are associated with generally high severity indices once zeroes are removed, this could indicate possible postmortem removal of dental calculus, provided that such postmortem removal was partial or targeted at certain teeth or quadrants, leaving little to no deposits in some areas and large deposits in other areas. Comparisons of trends within the two data sets could also prove very useful to see which trends are visible within both and which are not. Since calculus was typically not valued as a source of information in the past and was frequently removed to view the surface underneath, this may be the best strategy when post-excavation biases exist as it allows the researcher to analyse the data from multiple lines of inquiry. All future research and

excavations should regard dental calculus with the same care that they do the rest of the skeleton, to preserve the information it contains about past lifeways.

With respect to the microscopic analysis of dental calculus, this research demonstrates that it is feasible for dental calculus to provide new information on the lifeways of the ancient Cis-Baikal foragers but it also highlights a number of obstacles that must be considered and/or overcome. Results suggest a number of directions for future research. Of foremost importance is the compilation of a reference collection of relevant microparticles for the Baikal region, including starch grains, phytoliths, pollen, spores, and diatoms. This would involve sampling modern plant, water and soil sources within the Cis-Baikal, examining particles from residues on tools and ceramics, if available, and compiling information recovered from palaeoenvironmental and ethnographic studies on traditionally useful and available resources from each microregion. This would be a demanding undertaking but necessary in order to associate microparticles with particular foods or environmental sources.

Also of importance before undertaking further microparticle research on dental calculus is to develop methods that help minimize opportunities for contamination associated with handling and curation. In the future, all newly excavated skeletal material with the potential for sampling should be handled with powder-free gloves at all times and samples should be isolated as soon as possible to avoid environmental contamination. Storage containers that have been previously associated with food should never be used for skeletal material, and no remains should be stored and/or handled in environments where food processing, fungal growth or other sources of contamination are a factor. All sampling should occur in an isolated, contaminant-free facility and, if possible, extraction of dental calculus samples should be conducted in a positive pressure setting. Recently published work by Crowther et al. (2014) provides an excellent summary of problematic contamination sources and measures to eliminate these contaminants.

While contamination within the burial environment is unavoidable, and since previously excavated samples have already been exposed to contaminants, new techniques should be developed to better remove contaminants before sampling. While abrading with a tooth brush and sterile water is the standard now, studies, including this one, have documented particles associated with the burial environment that are embedded within calculus samples (e.g., Wesolowski et al. 2010). These particles get lodged in the slightly porous exterior of deposits and are difficult to remove (Wesolowski et al. 2010). Particles from the curatorial environment

can also become lodged in pores in a similar manner. If new techniques could successfully remove all such contaminants from the surface, many of the issues encountered during this study could be circumvented.

Once a reference collection has been created and potential for contamination has been more effectively controlled, the microscopic analysis of dental calculus has the potential to provide a wealth of information about past lifeways. While a large number of projects could branch from this preliminary work, four are suggested here. First, this research has established that various forms of damage are present on starch grains associated with *in vivo* calculus formation. An in-depth study on types of damage associated with food processing and preparation versus natural starch degradation could be conducted in an attempt to determine *in situ* evidence of food processing strategies in the Cis-Baikal. Combined with the identification of specific plant microparticles, archaeological material, and ethnographic studies in the region, this approach would provide significant new evidence on plant use in the Cis-Baikal, especially since the understanding of plant use in the region is quite limited at this time (Weber et al. 2002).

Second, another study could focus on distinguishing postmortem fungal activity from *in vivo* incorporation. One way to do this may be to analyze the deposit surface and exposed pores with SEM imagery for signs of fungal activity. Fungal activity associated with *in vivo* calculus formation would be very informative for both diet and possibly physiological stress, as highlighted in the study by Afonso-Vargas et al. (2015), where they found evidence of *in vivo* incorporation into mineralizing calculus of a pathological fungus typically associated with corn (see Chapter 5, Section 5.4).

Third, the potential for diatom recovery could help reconstruct foraging ranges if embedded diatoms can be associated with specific water sources. This is a fairly lofty goal, especially since the particles are only tentatively identified as diatoms and counts were extremely low, but it would be highly significant to BHAP research if successful, since it may be able to pinpoint with greater specificity where individuals and populations were traveling within the landscape. Because calculus severity seems to reflect microregional distinctions among populations and may in fact be linked to the water sources used by these populations, the two combined methods have great potential to provide significant information on foraging ranges in the Cis-Baikal. Both diatom and phytolith recovery may be more successful with SEM microscopy, such as was seen by Dudgeon and Tromp (2012).



Last of all, new research suggests that stable isotope analysis on dental calculus samples can provide meaningful information about archaeological populations. Scott and Poulson (2012) believe that the presence of nitrogen-bearing organic compounds in calculus indicates the potential for stable carbon and nitrogen analyses to be conducted. They sampled 58 medieval and post-medieval Basque and Spanish individuals from the Cathedral of Santa Maria and one precontact Alaskan Inuit individual as a comparison. The signatures obtained for the former were within the range of bone collagen isotopic data in Europe at the time, consistent with a diet based on temperate grasses with a moderate protein intake, while isotopic values obtained from the single Inuit individual were consistent with signatures from fingernails of Greenlandic Inuit, who consumed a diet rich in marine foods (Scott and Poulson 2012:1391). The authors state that the exact chemical form and sources of carbon and nitrogen preserved in the calculus are not understood at this time and more work needs to be done on their occurrence in calculus to comprehend what exactly is represented in the isotopic analysis. A comparison of bulk calculus samples and bone and dentin collagen, taken from the El Raval Mudéjar Medieval Cemetery (fifteenth century A.D.) in Eastern Iberia, led to the conclusion that these two media are not correlated at the individual level (Salazar-Garcia et al. 2014). They did produce broadly similar ratios at the population level nevertheless. And so, while calculus is not a replacement for bone and dentin collagen in terms of stable isotope analysis, calculus isotope analysis has the potential to provide valuable information in population studies (Salazar-Garcia et al. 2014).

Originally, stable isotope analysis was to represent a third technique within this present research project. Unfortunately, only a few samples were large enough to split for both microscopic and isotopic analyses, and ultimately the technique was withdrawn from the study in order to focus on the microscopic analysis of calculus deposits. As part of a future research project, it would be very interesting to see if stable nitrogen ratios in calculus samples correlate with dental calculus severity for riverine versus lakeshore populations, since it is thought that only lakeshore populations utilized seal (Weber and Bettinger 2010). Baikal seals are thought to have occupied the highest trophic level in the region and therefore have distinctly high  $\delta^{15}\text{N}$  ratios (Katzenberg et al. 2010). Consumers of seal meat would reflect these high stable nitrogen ratios and this has been noted for bone collagen samples from Cis-Baikal individuals from the Little Sea Region and also likely at Shamanka II (Katzenberg et al. 2010). If a correlation between  $\delta^{15}\text{N}$  ratios and dental calculus severity can be drawn, it would provide strong support

that consumption of seals was contributing to calculus formation. Also, since intensive stable isotope analysis has already been conducted on bone and tooth samples from these cemetery populations, it would be interesting to compare stable isotope ratios from these samples to those from calculus to determine if the two correlate or contradict each other; such a study would complement previous efforts (e.g., Scott and Poulson 2012; Salazar-Garcia et al. 2014) to use these multiple lines of data to determine what stable isotope ratios from calculus reflect and how they can be used as an additional line of archaeological data.

## **6.5 Concluding Remarks**

In conclusion, this research has established the value of dental calculus in reconstructing aspects of hunter-gatherer lifeways in the Cis-Baikal. Microregional variation in environmental conditions and resources appears to have been the dominant factor influencing calculus severity. The ratio of protein to carbohydrates in the diet does not adequately explain the trends observed, though it likely played a lesser role in calculus formation. Unknown non-dietary mouth use activities may also have affected calculus formation, particularly in the anterior dental quadrants of Shamanka II individuals. Microscopic analysis also provided direct evidence of potential plant processing of starchy foods, albeit in low quantities, and non-food particles incorporated into calculus through possible non-dietary mouth use, medicinal treatment, or the palaeoenvironment.

Dental calculus is a valuable tool once the mechanisms behind its formation are realized. This goes far beyond simple interpretations based on the effects of protein versus carbohydrates in the diet. The multicausal nature of dental calculus means that many potential variables need to be considered before drawing conclusions, with simple explanations and straight-forward associations being unlikely. However, with careful analysis, the study of dental calculus severity can provide an abundance of useful information on past lifeways. Microscopic inclusions in calculus, in particular, provide unique evidence of particles in direct contact with the individual during life. The biggest obstacle to microscopic studies on dental calculus is contamination. If contamination can be controlled, microparticles recovered can be confidently associated with plants and debris in contact with the individual during their life. This study has provided an in depth look at the strengths and benefits of using multiple techniques together to reconstruct aspects of Cis-Baikal hunter-gatherer lifeways, so long as limitations are considered, controlled, and eliminated when possible. These limitations were addressed and a number of solutions have been offered for future research.

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## **Appendix A: Raw Data for Macroscopic Analysis**

Table A-1 Tooth Surface Scores for Cis-Baikal Individuals

Cemetery	Burial	Element	RI1	RI2	RC	RP1	RP2	RM1	RM2	RM3	LI1	LI2	LC	LP1	LP2	LM1	LM2	LM3
Lokomotiv	L-1-1-1	maxilla			1,0,0,0	0,0,0,0	0,0,9,0	0,1,0,0	0,0,0,0				0,0,0,0	0,0,0,0	0,0,0,1			
		mandible		0,0,0,0	1,0,0,0	0,0,0,9	9,0,0,9	9,0,0,9	9,0,0,9				0,0,9,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	
	L-4-1-1	maxilla	0,0,0,0	0,0,0,0				0,9,9,9			1,0,0,1				9,9,9,0		0,0,9,9	
		mandible			0,0,0,9	9,0,1,9	0,9,9,9	0,0,0,9	0,0,1,0						1,0,1,1	1,0,1,0		
	L-7-1-1	maxilla		9,0,0,0				0,9,0,9	1,0,0,9			9,0,0,9	9,0,0,9	0,0,0,9	9,1,0,9		9,0,0,0	0,0,0,0
		mandible	0,0,0,0	0,9,9,0	0,0,9,9	9,0,0,0	0,0,0,0				9,0,0,0	0,0,0,0	0,0,0,0	0,0,0,9	9,0,0,9	9,0,0,0	1,0,0,0	0,0,0,9
	L-8-1-1	maxilla		9,9,0,9	0,0,0,0	0,0,0,0	9,9,9,0	0,9,0,0	0,0,0,0				9,9,9,0	0,9,9,9	0,9,0,0	9,9,9,0		
		mandible	0,0,0,0	9,0,0,9									9,0,9,0			0,0,0,0	0,0,0,0	
	L-9-1-1	maxilla	1,0,0,1	1,1,0,1	0,0,0,1	0,2,0,1	1,2,0,1				1,0,0,1	1,0,0,0	2,1,1,1	9,1,9,1	2,2,0,0	1,1,9,9		
		mandible																
	L-10-1-1	maxilla	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,9	9,0,0,9	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0
		mandible																
	L-10-2-1	maxilla	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	1,0,0,0	1,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	9,0,0,0	0,0,0,0	0,0,1,0	0,0,0,0	
		mandible		0,0,0,0	0,0,0,0	0,0,0,0					1,0,0,1	0,0,0,0	0,0,0,0	0,0,0,0	9,0,0,0	0,0,9,0	0,0,9,0	0,0,9,0
	L-11-1-1	maxilla	0,0,0,0	9,0,0,9	9,0,0,0	0,9,0,0	9,0,0,9	9,0,0,0	0,0,0,0				0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,9	9,0,0,9	
		mandible	0,0,0,0	0,0,9,0	0,0,0,0	0,0,0,0	0,0,0,9	9,0,0,9	9,0,0,9	9,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,9,0			
	L-13-1-1	maxilla	0,0,0,0		1,0,0,0	0,0,0,0	0,0,0,0	9,0,0,0	0,0,0,0	1,0,0,0	1,0,9,0	9,0,0,0	1,0,0,0	0,0,9,0	0,0,0,0	0,0,0,0	0,0,0,0	
		mandible	2,0,0,2	9,0,9,1	1,0,0,1	1,0,0,1	0,0,0,0				2,0,0,9	2,0,1,1	1,0,9,1	1,0,0,0		9,9,9,0	9,9,0,9	0,0,0,0
	L-14-3-1	maxilla	0,0,0,0					0,0,0,0			0,0,0,0		0,0,0,0	0,0,0,0		0,0,0,0		
		mandible																
	L-15-1-1	maxilla			0,0,0,0	0,9,9,0	0,1,0,9	0,1,0,9	1,9,9,9	0,0,0,0			9,9,0,0	0,0,0,0	0,0,0,0	1,9,0,0	0,1,0,0	9,0,0,0
		mandible	2,0,0,1		0,0,0,0	0,0,2,0	1,0,9,0	0,0,0,0			1,0,1,2			1,0,3,0		0,0,0,0	0,0,0,0	
	L-16-1-1	maxilla	2,0,9,1	2,2,0,1	1,0,0,1	1,1,0,2	1,2,1,2	2,9,1,9	9,1,1,1		2,0,0,1	2,0,0,1	2,1,1,9	1,1,1,1	2,1,1,9	9,2,1,2	9,1,0,1	
		mandible	1,1,1,1	2,2,0,2	2,0,1,1	1,0,2,2	2,1,3,2			1,0,1,1	1,9,9,0	2,0,9,9		1,1,2,1	1,2,1,1	1,1,1,1	9,9,1,0	
	L-18-1-1	maxilla					0,0,0,0								0,0,0,0		9,9,0,9	
		mandible																
	L-19-1-1	maxilla			0,0,0,0	0,0,0,0	0,9,0,1	1,0,9,9	9,0,1,1	9,0,0,0			9,9,9,0	0,0,9,0			0,0,0,0	0,0,0,0
		mandible			9,9,0,0		0,9,1,1	1,1,2,1	0,0,2,1		0,0,0,0			0,0,0,0	0,0,9,0			

Cemetery	Burial	Element	RI1	RI2	RC	RP1	RP2	RM1	RM2	RM3	LI1	LI2	LC	LP1	LP2	LM1	LM2	LM3
	L-20-1-1	maxilla		0,0,0	0,0,0	0,0,0	0,0,0	0,0,0				0,0,0	0,0,0,1	1,0,0,1	0,0,9,0	0,0,0,0		
		mandible	0,1,0,0	2,1,0,1	9,1,0,0	1,1,1,0	0,1,1,0				1,1,0,0	0,1,0,0	0,1,0,1	1,1,1,0	1,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0
	L-20-2-1	maxilla	0,1,0,0		0,2,0,0	0,2,0,0	0,9,0,9	9,3,1,9	9,9,0,1			0,1,0,9	9,1,0,0	9,2,0,0	0,2,0,1	9,3,0,9	9,2,0,9	1,3,0,3
		mandible		0,1,0,9	9,1,0,0	0,1,0,0	1,0,0,0		9,0,0,9	9,9,0,0		0,0,0,0		1,2,0,0				0,1,9,9
	L-21-1-1	maxilla											0,0,0,0					
		mandible	0,0,0,0		0,0,0,0	0,0,0,0	0,0,0,0				0,0,0,0						0,9,0,0	0,0,0,0
	L-23-1-1	maxilla	0,0,0,0	0,0,0,0	9,0,0,0	9,0,0,0	0,9,9,0	0,0,0,1	0,0,0,0	0,0,0,0		0,0,0,0	0,0,0,0	0,0,0,0	1,0,0,0	9,0,0,1	1,0,0,0	0,0,0,0
		mandible	1,0,0,0	0,0,0,0	0,9,0,0	1,0,2,0	0,0,2,0			0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0		
	L-24-3-1	maxilla						9,0,0,0										
		mandible						0,0,0,0								0,0,1,0		
	L-24-4-1	maxilla	0,9,0,9	9,0,0,0				0,0,0,9			0,9,0,0	0,0,0,9				0,0,0,0		
		mandible	0,0,0,0	0,0,0,0				0,0,0,0			0,0,0,0	0,0,0,0						
	L-25-1-1	maxilla																
		mandible			0,0,9,9	0,9,0,0		9,0,9,9	0,9,9,0	0,9,9,9			0,0,0,0	0,9,1,0	0,0,1,9			
	L-25-5-1	maxilla				0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0			0,0,0,0				0,9,0,0	0,0,9,9
		mandible																
	L-27-1-1	maxilla	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0			0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0		
		mandible		0,0,0,0	0,9,9,9			0,0,0,0					0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	
	L-28-1-1	maxilla	0,9,0,9	9,0,0,9	0,9,0,0	0,0,0,0	0,9,9,0					9,9,0,0	9,1,0,1	9,9,0,0	0,9,9,1	1,1,9,9	9,0,9,9	
		mandible	1,0,0,2	2,0,9,2	9,1,9,1	0,0,1,0	0,9,0,0	9,1,1,0	0,9,1,0		2,1,0,1	2,0,0,9	1,0,9,9	0,9,1,0	0,0,0,0	0,0,0,0		
	L-30-1-1	maxilla	9,0,0,0	1,0,9,1	0,9,0,0	0,9,9,0	0,9,9,0	0,0,9,0			1,9,0,0	0,0,9,9	0,9,0,0	0,9,0,0	0,9,9,0	0,0,9,0	0,0,9,0	
		mandible			9,1,9,9	0,1,0,1	1,0,9,1				0,9,9,0	9,0,0,0	0,9,0,9	1,0,1,1	2,9,2,0			
	L-30-2-1	maxilla		0,0,0,0			0,9,0,0			0,0,0,0		9,1,0,9		0,0,9,9	0,9,9,0	0,1,9,0		0,0,0,0
		mandible		9,0,0,0	1,0,0,0	0,0,9,0	1,0,9,1				0,9,9,9	0,0,0,0	1,0,0,0	0,0,9,9				
	L-31-1-1	maxilla			0,0,0,0	0,0,9,1	0,0,0,0		1,0,9,0					0,0,0,0	0,0,1,0			
		mandible																
	L-31-2-1	maxilla	1,0,0,1	0,0,0,1			1,0,0,1	1,1,0,0	0,0,0,0		1,0,0,1	1,0,0,0	1,0,0,0	0,0,0,1	1,0,0,0	1,1,0,0	1,0,0,0	
		mandible	2,0,0,1	1,0,0,2	1,0,0,1	0,0,1,1	1,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	1,0,2,1	1,0,0,1	1,0,1,0	1,0,1,0				
	L-33-1-1	maxilla			9,0,0,1	0,1,0,0	0,9,0,1	1,1,0,1	0,0,0,1	0,0,0,0			0,0,0,0	1,0,0,0	0,0,0,0	0,9,9,0	0,0,0,0	0,0,0,0
		mandible				0,0,0,1	0,0,0,1	0,0,0,0	0,0,1,0	0,0,1,1		0,0,0,1	1,0,0,1	1,0,0,1	1,0,0,1			

Cemetery	Burial	Element	RI1	RI2	RC	RP1	RP2	RM1	RM2	RM3	LH1	LI2	LC	LP1	LP2	LM1	LM2	LM3
	L-34-1-1	maxilla	0,0,0,9	0,0,0,0	0,9,0,0	0,0,9,0	0,9,9,0	1,9,0,0	0,9,9,9	9,0,0,0	0,0,0,0		0,9,0,0	0,9,0,0	0,0,9,0	0,9,9,0	0,0,9,9	1,0,0,1
		mandible	9,0,9,9	0,0,0,0	0,0,0,0	0,0,0,0	0,9,0,0				0,0,0,0	9,0,9,0	0,0,0,0	0,0,0,0	0,9,9,0	0,9,0,9	9,9,0,0	9,0,0,0
	L-35-1-1	maxilla	1,9,0,9	0,1,0,0	0,0,0,0	1,0,0,1	1,0,0,0	1,0,9,1	1,0,0,0	1,0,1,9	1,0,0,2	2,2,0,1	1,9,0,0	9,1,0,1	9,0,9,9	2,1,9,1	2,9,0,9	1,0,1,1
		mandible	2,0,1,2	1,0,0,1	1,0,1,1	2,1,1,1	1,0,1,1	1,0,0,0	0,9,9,9	0,9,1,9	2,0,0,2	2,0,0,2	2,0,1,2	1,9,1,2	2,0,1,1			
	L-37-1-1	maxilla																
		mandible				9,9,0,9	0,9,9,9	9,0,9,9	9,0,0,0			9,0,0,1	9,0,0,0	0,0,1,0				
	L-38-2-1	maxilla		0,0,0,0	1,0,0,1	0,9,0,0	0,9,0,0	1,0,0,0	0,0,0,0	9,9,0,0	1,0,1,1	0,0,0,0	1,0,0,0	1,1,0,1	1,1,0,0	1,1,0,0	0,0,0,0	0,0,0,9
		mandible	9,9,1,9	0,1,0,1	1,0,1,1	1,0,2,0	1,9,1,0	0,0,1,0				9,1,0,0	0,0,0,0	1,0,0,1	1,0,9,0			
	L-39-1-1	maxilla	0,0,0,1	0,0,0,0	0,0,0,0			0,0,0,0	0,0,0,0		0,0,0,0		0,0,0,0	0,0,0,0		0,0,9,9	0,0,0,0	
		mandible		1,0,9,0	1,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0			0,0,0,9	0,0,0,0	0,0,0,0	0,0,9,0			
	L-41-1-1	maxilla		0,0,0,0		0,0,0,0		0,0,0,0	9,0,0,0					0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	
		mandible				0,0,9,0	0,0,0,0		0,0,0,0					1,0,1,0	0,0,0,0			
	L-41-2-1	maxilla																
		mandible	0,0,0,0					0,0,0,0								0,0,0,0		
	L-42-1-1	maxilla	9,0,0,0			0,0,9,9	0,9,0,0	0,0,1,0	1,0,0,0		9,0,0,9	0,0,0,0	0,0,0,1	0,9,0,1	0,9,0,1	0,9,0,1	0,9,9,9	
		mandible	0,0,0,0	0,0,0,0	0,9,0,0	0,0,9,0	1,0,1,1				0,0,0,0	0,0,0,0	0,9,0,0	0,0,9,0	0,0,9,0	0,0,0,0	0,0,0,0	
	L-43-2-1	maxilla		0,0,0,0	0,0,0,0	0,1,0,0	1,0,0,1	9,0,0,1	1,0,0,0	0,0,0,0		0,0,0,0	0,0,0,0			0,0,0,1	1,0,0,0	0,0,0,0
		mandible		0,0,0,1	1,0,0,0	0,0,0,0	0,0,0,0							0,0,0,0	0,0,0,0	9,0,1,0	0,0,0,0	
	R-2-1-1	maxilla	0,0,0,0	0,0,0,1	1,0,0,1	1,1,0,1	1,1,0,9	9,1,1,0	0,1,1,0	0,1,0,0	0,0,0,0	0,0,0,9	9,0,0,0		1,0,0,1	9,1,0,9	9,0,0,9	9,1,0,0
		mandible	1,0,0,1		0,0,0,1	1,0,0,1	1,0,1,1				1,0,1,1	1,0,1,9	9,0,2,0	0,0,2,0	0,0,2,1	9,1,2,9	9,0,3,0	
	R-7-1-1	maxilla	0,1,0,0	0,0,0,0	0,0,0,0	9,9,0,0	0,0,0,9	0,0,0,0	0,0,0,0		9,0,0,9	1,0,0,9	0,0,0,1	0,9,0,0	0,9,0,0	0,9,0,0	9,0,0,9	
		mandible			0,0,0,0	1,9,9,9	1,9,1,0	0,0,0,9	0,9,0,0				0,0,9,9	0,0,9,1	1,0,2,0			
	R-9-1-1	maxilla						0,9,0,0								0,9,0,0		
		mandible						0,0,0,0			0,0,0,0							
	R-11-1	maxilla	1,0,0,1	1,1,0,1	1,0,0,0	1,1,0,1	1,2,0,0	9,9,0,0	0,0,0,0		1,0,0,1	1,1,0,1	2,1,0,0	1,1,1,1	1,1,1,1			0,0,0,0
		mandible	2,1,3,1	2,1,0,1	1,0,1,1	1,0,2,1	0,0,1,0	0,9,0,0	0,0,0,0	9,0,0,9	2,1,2,1	2,1,0,1	1,0,1,1	2,0,1,1	1,0,1,0	0,0,0,0	1,0,1,0	0,0,0,0
	R-12-1	maxilla	0,0,0,0	0,0,0,0				0,0,0,0			9,0,0,9	0,0,0,9				0,0,0,0		
		mandible	0,0,0,0	0,0,0,0		0,0,0,0		0,0,0,0			0,0,0,0	0,0,0,0						
	R-13-1	maxilla						0,9,0,0								0,9,9,9		
		mandible	9,9,0,0								9,0,0,0							

Cemetery	Burial	Element	RI1	RI2	RC	RP1	RP2	RM1	RM2	RM3	LI1	LI2	LC	LP1	LP2	LM1	LM2	LM3
Shamanka II	R-14-1	maxilla			1,0,0,1	0,0,0,1	0,0,0,1	0,0,9,0	0,0,0,0		0,0,0,0			9,1,9,1	1,0,1,0	1,9,9,1		
		mandible	3,0,1,2	3,0,0,2		1,0,2,1	1,0,2,1	1,0,2,0	1,0,1,0		2,0,0,3			2,1,1,1	2,0,2,1			
	R-15-1	maxilla	0,1,0,0	0,1,0,0	0,1,0,0	1,0,0,0		0,0,0,1	0,0,0,0	0,0,0,0			0,0,0,0	0,0,0,0	0,0,0,0			
		mandible	9,2,0,1	1,1,0,1	1,9,9,1	1,1,1,0	0,0,1,0	0,0,0,0	1,0,0,0		1,1,0,9	1,1,0,1	1,0,0,1	1,0,1,0	1,0,1,1	1,0,1,1	1,0,0,0	1,0,0,0
	R-15-2	maxilla							0,0,9,0			9,9,0,9	9,0,0,9	0,0,9,9			1,0,0,0	
		mandible	9,0,0,9	9,0,0,0	0,0,0,0			9,0,0,9				9,0,0,9		9,0,0,0	0,9,0,0	0,0,0,9		
	6	maxilla	1,1,0,0	0,1,0,0	0,1,0,0	0,1,0,0	0,0,0,0	0,1,0,0	0,0,0,0		1,1,0,1	1,1,0,0	0,1,0,0	1,1,0,0	0,1,0,0	1,1,0,1	0,0,0,0	0,0,0,0
		mandible	1,2,0,1	0,1,0,1	1,2,0,0	1,1,1,1		1,0,1,1			1,2,0,1	2,1,1,1	1,1,0,1	1,1,1,1	1,1,1,1			0,0,0,0
	7	maxilla	1,0,0,0	1,0,0,1	0,0,0,0	0,0,0,0	1,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,1	1,0,0,0	0,0,0,1	0,0,0,0	1,0,0,1	1,1,0,0	0,0,0,0	0,0,0,0
		mandible	1,1,0,1	1,0,0,2	1,0,0,0	0,0,0,0	0,0,0,0				2,1,0,1	1,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0		0,0,0,0	0,0,0,0
	8-1	maxilla			1,1,0,1	9,0,9,1					1,0,0,0	0,1,0,0			1,1,0,0	1,2,0,1	1,0,0,0	0,0,0,1
		mandible	1,1,0,0	1,1,0,1	1,0,0,1	2,1,0,0	2,0,1,1						0,2,0,0	1,1,0,1	1,0,1,1	0,0,1,1	0,0,1,0	
	9	maxilla			0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0		0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,9	
		mandible			1,0,1,0	1,0,1,1	0,0,1,0					1,0,0,9	0,0,0,0	1,0,1,0	0,0,0,0	0,0,0,0	0,0,9,9	
	10	maxilla																
		mandible						1,0,0,0	0,9,9,9	1,1,1,0						1,0,1,0	0,0,1,0	0,0,1,0
	11-1	maxilla	0,0,0,0		0,0,0,0		1,0,0,0	1,0,0,0	0,0,0,0	0,0,0,0			0,0,0,0	0,0,0,0		0,1,0,0	0,0,0,0	0,0,0,0
		mandible	0,0,0,0		0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0			0,0,0,0	0,0,0,0	0,0,0,1			
	11-2	maxilla		2,0,0,2	2,1,0,1	1,1,0,0	1,0,0,1	2,1,0,0	1,0,0,1		2,0,0,2	2,0,0,2	2,1,0,2	2,1,0,1	1,0,0,1			
		mandible																
	12	maxilla																
		mandible		2,0,1,1	2,1,1,1	1,1,2,1	1,1,1,1	1,0,1,1	1,0,0,1				1,1,1,1	1,1,2,1	1,0,1,1			0,0,0,0
	13-3	maxilla						0,0,0,0	0,0,0,0		0,0,0,0			0,0,0,0	0,0,0,0	0,0,0,1	1,1,0,0	
		mandible						0,0,0,0	0,0,0,0									
	14-1	maxilla		1,1,0,1	1,0,0,1	1,0,0,9	1,0,0,0	1,0,0,0	0,0,0,0	0,0,0,0	1,0,0,2	1,0,0,1	1,0,0,0	1,1,0,1	9,0,0,0	1,0,0,0	1,0,0,0	0,0,0,0
		mandible	3,1,0,2	2,1,0,2	9,1,9,1	1,1,1,1	1,0,0,1				1,1,0,2	1,1,0,2	1,1,0,2	2,1,0,2	1,0,0,0	1,0,0,0	0,0,0,0	1,0,0,0
	14-2	maxilla	0,1,0,0		1,0,0,0		0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0			0,0,0,0	0,0,0,0	1,0,0,1	0,0,0,0	0,0,0,0	0,0,0,0
		mandible	0,0,0,0		0,0,0,0	0,0,0,0	0,0,9,0					0,0,0,0	0,0,0,1	1,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0
	15	maxilla			3,1,0,1	0,1,0,1	2,1,0,0	1,3,0,2					1,0,0,1	1,0,0,1	1,0,0,2	2,3,1,0		
		mandible		1,0,0,1	9,0,0,2	3,1,2,9	3,1,2,2					2,1,0,3	3,1,1,1	2,2,1,1	1,3,2,3	1,3,2,2	1,9,1,0	0,9,1,9



Cemetery	Burial	Element	RI1	RI2	RC	RP1	RP2	RM1	RM2	RM3	LI1	LI2	LC	LP1	LP2	LM1	LM2	LM3
	17-1	maxilla	9,0,9,0	0,0,0,1	1,0,0,1	0,1,9,0	1,0,0,0	0,3,0,0		0,0,0,0	1,0,9,0	0,0,0,1	0,0,0,9	0,1,9,1	1,0,0,0	0,9,9,0	1,1,0,0	0,0,0,0
		mandible	1,0,0,1	2,0,1,1	1,0,0,1		1,0,9,1					1,1,1,1	2,0,0,1	1,0,0,0	1,0,0,0	9,1,9,0	0,9,0,0	0,0,1,1
19	maxilla						2,1,0,0	9,2,0,0	1,0,0,1				0,1,0,1	1,2,0,1	1,9,0,1	0,2,0,1	0,1,0,0	
		mandible									1,0,0,1			1,1,1,1	1,1,1,1	0,1,0,0	0,0,1,0	0,0,1,0
21-1	maxilla	0,0,0,1	0,0,0,0	1,1,0,1	1,0,0,0	0,0,0,0	0,1,0,1	1,1,0,0	0,0,0,0	0,0,0,0	1,0,0,9	0,1,0,1	1,1,0,1	0,2,0,0	0,9,9,9	2,1,0,0	0,0,0,0	
		mandible		1,0,0,3		1,0,1,0	0,0,1,0				1,0,9,2	1,0,0,1	9,9,9,9	1,9,0,1	1,0,2,1	0,0,9,0	1,0,9,0	0,0,9,0
21-2	maxilla	1,1,0,1	1,0,0,0	1,1,0,0	0,1,0,1	0,1,9,0	0,9,0,1	9,0,0,0	0,0,0,1	1,1,0,0		1,1,0,1	1,1,0,1	0,0,0,0	9,2,0,9	9,0,0,0	0,0,0,0	
		mandible	2,1,0,1	9,0,9,1	2,1,1,0	1,1,1,1	1,1,2,1				2,2,1,2	9,1,1,1	2,1,0,1	1,1,1,1	1,1,0,1	0,1,1,9	9,0,1,0	0,0,0,0
21-3	maxilla	0,0,0,0	0,0,0,0		0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0		0,0,0,0	0,0,0,0	9,9,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	
		mandible	1,1,0,1	1,0,0,1	1,0,0,9	0,0,0,0	1,0,1,0		0,0,0,0		1,0,1,1	1,0,0,0	1,0,0,9	0,0,0,0	1,0,0,0			
22	maxilla		1,0,0,0		1,0,0,1	1,0,0,1	0,0,0,0	1,0,0,0	0,0,0,0			1,0,0,1	0,0,0,1		0,0,0,0	0,0,0,0		
		mandible		1,0,1,1	2,0,1,0	0,0,0,0					1,0,1,3	2,0,1,1	0,0,1,1	0,0,1,0	0,0,0,0		0,0,0,0	
23-1	maxilla	0,0,0,0	0,0,0,0		1,0,0,0	0,1,0,0	1,2,0,1			0,2,0,1	0,1,0,0		0,0,0,0	0,0,0,0	0,9,0,0	1,2,0,1		2,2,0,2
		mandible	1,0,0,0	0,1,1,9	9,0,1,1	1,0,1,1	1,1,9,0				1,1,1,1	1,1,0,1	1,1,1,1	9,1,9,1				
24-1	maxilla			0,0,1,9	0,1,1,9	1,0,1,9	0,1,1,9	1,0,1,0	1,2,1,2				1,1,0,0	1,1,0,1	1,1,1,1	1,2,0,0	2,1,0,0	
		mandible			0,0,0,1	1,1,2,1	1,1,2,1						0,2,0,0	1,0,2,1	0,1,2,1	0,0,9,1	0,1,9,1	0,0,0,0
26-3	maxilla							1,0,0,0								0,0,0,0		
		mandible	1,0,0,0															
29-1	maxilla	0,0,0,1	1,0,0,1	1,0,0,1	1,1,0,0	1,0,0,1	1,1,0,0	0,0,0,0	0,0,0,0	1,0,0,1	1,0,0,0	1,0,0,0		1,1,0,0	1,1,0,0	1,0,0,0		
		mandible	9,0,0,1	1,0,1,1	1,1,1,0	1,0,1,0	1,0,0,0	1,0,1,0	0,0,1,0		1,0,1,9	0,0,0,1	1,0,1,1	0,0,1,0	0,0,0,0			
30	maxilla	1,1,0,2	2,1,0,1	2,1,0,1	1,1,0,2	1,1,0,2	1,2,1,1	1,2,0,1		2,1,0,2	2,2,0,1	9,9,9,1	0,1,0,1	0,1,0,0		1,2,0,1	1,1,0,0	0,0,1,0
		mandible	2,1,1,3	1,1,1,1	9,2,0,2	2,2,1,1	1,1,1,1	2,3,1,2		1,1,0,0	1,9,1,0	2,9,0,2	1,2,1,2	2,2,1,2	1,1,0,1			
32	maxilla		0,0,0,0		1,1,9,1	2,1,0,2						0,0,0,0	2,0,0,2	1,0,0,9	9,0,1,0	0,1,0,1	1,2,0,0	
		mandible	2,1,0,2	1,0,1,9	3,1,2,2	3,0,2,2	2,2,2,1	2,1,2,1			9,0,0,1	2,0,0,9	2,0,1,9		0,1,1,1			
33	maxilla	0,0,0,0									1,1,0,0	0,0,0,1	0,0,0,0					
		mandible	3,1,0,2		1,0,0,1		1,1,2,1				3,2,0,3	3,1,0,2	2,1,0,1	1,1,2,2	1,0,1,1	1,0,1,1	0,0,0,0	1,0,0,0
34	maxilla	2,0,0,0	1,0,0,0	1,0,0,1	1,0,0,0	0,1,0,0		0,0,0,0		1,0,0,1	2,1,0,0	0,0,0,0		1,0,0,1	0,0,0,0	0,1,0,0	0,0,0,0	
		mandible	1,0,9,9	0,0,9,2	2,1,1,2	2,1,2,1	1,1,0,1		0,0,9,0	0,0,1,0	1,1,0,0	0,0,9,2	2,1,1,1	1,1,2,2	2,0,2,1	1,0,1,9		
35-1	maxilla		0,0,0,0	0,0,0,0	0,0,0,0	1,1,0,1							1,0,0,0	0,0,0,1	1,0,0,0	0,9,0,9	1,1,0,0	0,0,1,1
		mandible																

Cemetery	Burial	Element	RI1	RI2	RC	RP1	RP2	RM1	RM2	RM3	LI1	LI2	LC	LP1	LP2	LM1	LM2	LM3
	36	maxilla	0,0,0,0			1,1,0,1	1,1,1,0	0,0,1,1	1,0,1,0		1,0,0,0	0,0,0,0	0,0,0,1	0,0,0,1	9,9,0,9	1,0,1,1	1,0,0,0	
		mandible	3,2,1,3	2,2,2,2	2,1,2,2	1,1,1,1	1,2,2,0	0,2,2,1		0,1,1,0	2,1,1,3	3,1,1,1	2,1,1,1	1,1,3,2	2,1,1,1			
	39	maxilla	0,1,0,0	1,1,0,0	1,1,0,1	1,0,0,0	1,9,0,0	1,9,0,0	0,0,0,0	0,0,0,0	0,0,1,0	0,1,0,0	0,1,0,0	0,0,0,1	1,0,0,0	0,1,0,0	0,1,0,0	0,0,0,0
		mandible		1,0,0,0	0,0,0,0	0,0,0,1	0,0,1,0					0,0,0,0	0,0,0,1	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0
	41	maxilla	1,0,0,0		0,1,0,0	0,0,0,0		0,0,0,0	0,1,0,0		1,1,1,1	0,1,0,1			1,0,0,0	0,0,0,0	0,0,0,1	0,0,0,0
		mandible	2,1,1,1	2,1,1,1	1,1,1,1	0,0,1,0	1,0,1,0				2,1,1,1	1,1,2,1	1,1,1,1	1,0,2,1	0,0,0,1	0,0,9,0	0,0,1,0	0,0,0,0
	42-1	maxilla		1,0,0,0	0,0,0,0					0,0,0,0	0,1,0,0	0,0,0,0	0,0,0,0		0,0,0,0	9,9,0,0		
		mandible	0,1,0,0	0,0,0,0	0,0,9,0			1,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	9,0,0,0	0,0,9,0	0,0,0,9	0,0,0,0			
	44-1	maxilla	1,1,1,1	0,1,1,0	1,2,0,1	0,3,0,2	1,1,0,1				1,1,0,0	0,1,1,0						
		mandible																
	45	maxilla		0,0,0,0	0,0,0,0	0,0,0,0	0,1,0,0	0,1,0,1	0,0,0,1			0,0,0,0	0,0,0,0		0,0,9,0	0,1,0,0	0,0,0,1	
		mandible	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,1	0,0,0,0			0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,9	0,0,1,0			
	46	maxilla		9,2,0,1		1,1,9,0	9,2,9,0	0,3,0,1			0,2,0,0			1,3,0,0	2,3,0,1			
		mandible	3,2,2,3	3,3,1,2	2,2,0,0	0,1,1,1	2,2,2,2	2,1,2,2			3,3,1,3	9,9,1,9	2,1,2,1	1,1,2,2	2,1,2,2			
	47	maxilla	0,1,0,0	0,0,0,0	0,1,0,0	0,1,0,0	0,1,0,0	0,1,0,1	0,1,0,0	0,0,0,0	0,1,0,0	0,0,0,0	1,1,9,0	0,1,9,0	1,1,9,0	1,2,9,1	1,0,9,9	0,0,0,0
		mandible	3,1,1,1	2,2,1,2	1,1,0,0	1,1,0,0	1,2,2,0				2,1,1,3	2,1,1,2	1,1,1,1	0,0,0,0	1,0,1,0	0,0,2,0	0,0,1,0	0,0,0,0
	48-1	maxilla		0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,1,0,1	9,9,0,0	0,0,0,0	0,1,0,0		0,0,0,0	0,0,0,0	0,0,0,0	0,1,0,0	1,1,0,0	
		mandible		0,0,0,0	1,0,0,0		0,0,0,0	0,0,0,0	0,0,0,0		9,0,0,0	0,0,0,0	0,0,0,1	0,0,0,0	1,0,9,1			
	49-1	maxilla	0,0,0,0	0,0,0,0	0,1,0,0	0,0,0,0	0,1,0,0	1,2,0,1	0,0,0,0	0,0,0,0		0,0,0,0	0,1,0,0	0,0,0,0	0,1,0,0	9,1,0,9	0,0,0,0	0,0,0,0
		mandible	1,0,0,1		0,0,0,0	0,0,0,0	0,0,0,0				2,0,0,1	1,0,0,1	1,0,0,0	1,0,0,1	1,0,1,0		0,0,1,0	0,0,0,0
	50-1	maxilla	0,1,0,2	2,2,0,1	2,1,0,1	1,1,0,1	1,1,0,0	0,0,0,1	0,0,0,1	0,0,0,1	2,1,0,2	1,1,0,1	2,1,0,1	1,1,0,0		0,1,9,0	0,0,0,0	0,0,0,1
		mandible	3,2,2,2	3,2,1,2	3,2,2,2	1,0,2,1	1,1,2,1				3,1,2,2	1,2,1,2	2,2,2,2	2,1,1,1	1,1,1,0	1,0,1,0	9,0,9,0	0,1,0,1
	50-2	maxilla				1,0,0,1	0,0,0,0	0,1,0,0			0,1,0,0		0,0,0,1	1,0,0,1	1,0,0,0	0,1,0,0		0,0,0,0
		mandible	2,1,0,1	1,1,1,1	1,0,1,0	0,0,0,1	0,0,1,1				2,1,0,1	2,0,0,1	2,1,0,1	0,0,0,1	1,0,0,0	0,0,0,0		
	51	maxilla	1,0,0,9	9,1,0,1	0,1,0,0	0,1,0,0	0,1,0,0	1,1,0,1	0,9,0,0		1,0,0,1	1,0,0,0	1,0,9,9	0,0,0,1	1,0,0,1	1,1,0,0	1,1,0,0	
		mandible	2,0,1,1	9,1,1,1	1,1,1,1	0,1,1,0	0,0,0,0				0,1,1,0	1,0,2,2	0,0,0,0	0,1,1,0	0,0,1,0	1,0,1,0	0,0,0,0	
	53-1	maxilla	0,0,0,0		0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0		0,0,0,0		0,0,0,0	1,0,0,0	0,1,0,0	0,0,0,0	0,0,0,0
		mandible	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0				0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0
	54-1	maxilla	1,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,1,0,0	0,0,0,0	0,1,0,0		0,1,0,0	1,0,0,1	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0
		mandible			1,0,0,0	0,0,0,0	0,0,0,0				1,0,0,1	1,1,0,0	0,1,1,0	0,0,1,0	0,0,0,0	0,0,0,0	0,0,0,0	

Cemetery	Burial	Element	RI1	RI2	RC	RP1	RP2	RM1	RM2	RM3	LI1	LI2	LC	LP1	LP2	LM1	LM2	LM3
	55-1	maxilla			1,0,1,1								1,1,1,0	1,1,1,1	1,2,0,1			
		mandible	9,9,1,9		2,2,1,1		2,1,1,1	1,1,1,1	1,1,1,1	1,1,1,1	2,1,0,1	2,2,1,3	2,1,1,2	3,2,1,2	2,2,2,2			
	55-2	maxilla						0,0,0,0										
		mandible														0,0,0,0		
	56-2	maxilla	0,0,0,0					0,0,0,0			0,0,0,0	0,0,0,0						
		mandible																
	57-1	maxilla																
		mandible				1,0,0,1		0,0,0,0						1,0,0,1	1,0,1,1			
	57-2	maxilla	0,0,0,9	0,1,0,0	1,1,0,1	1,0,0,0	0,0,0,0	0,1,0,0	0,0,0,0	0,0,0,0	1,0,0,0	0,1,1,1	9,0,0,1	0,0,9,1	0,0,0,1	0,1,0,1		0,0,9,0
		mandible	2,0,1,2	0,0,1,2	2,0,0,0	0,0,1,0	0,0,0,0				2,0,1,2	2,0,1,2	1,1,0,2	1,1,1,1	0,0,1,1	1,1,0,1	0,0,0,0	0,0,0,0
	58-1	maxilla		0,1,0,0	1,2,0,0		1,1,0,2	1,3,0,2	1,1,9,0	1,2,1,1	9,1,0,0	0,1,0,0	0,9,0,0	9,1,9,9	0,3,0,2			1,0,0,0
		mandible		0,1,1,2	2,0,0,2	2,2,0,2	0,1,9,9					0,0,0,0	0,1,0,1	1,1,1,1	2,1,0,0			
	59-1	maxilla		0,2,0,0		0,0,0,0	0,2,1,1	1,1,1,0		0,1,1,1	1,2,1,1	2,2,0,0	1,2,1,1			2,1,0,0	1,1,0,0	
		mandible	0,1,1,0		0,1,0,1					1,0,1,1			0,0,0,0	1,0,1,1				
	59-2	maxilla	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,1,0,1				0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0				
		mandible	0,0,0,0	0,0,0,1														
	60-2	maxilla																
		mandible	1,0,0,0	0,1,0,2	9,1,0,2	1,1,2,2	1,0,1,0	0,1,0,1	0,1,1,0	0,0,1,0	0,1,0,1	0,2,0,2		0,0,2,1	1,0,0,0			
	61-1	maxilla	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,9,1	1,0,0,0		0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	
		mandible	1,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0		0,0,0,0	0,0,0,0		0,0,0,1		0,0,0,0	0,0,0,0				
	61-2	maxilla			1,0,0,0	1,1,0,0	0,0,0,0	0,1,0,0	0,0,0,1		1,1,0,0		1,0,0,0	1,1,9,1	2,1,9,0	9,1,9,1	1,9,0,0	
		mandible		1,1,0,0	1,1,0,1	2,1,9,1	1,1,9,1	1,1,0,0	0,0,0,0		1,0,0,1		9,1,0,2	1,1,0,0				
	62-5	maxilla	0,0,0,0	1,0,0,0	0,0,0,0		0,0,0,0	0,0,9,0	0,0,0,0	0,0,0,0	0,0,0,0		0,0,0,0		0,9,9,9	9,1,0,0	0,0,0,0	
		mandible	0,0,0,0		0,0,0,0	0,0,0,0	0,0,1,0						0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,9		
	63-1	maxilla	2,1,0,1	1,1,0,1	1,1,0,1		0,1,0,0	0,2,0,0	1,0,0,0	0,0,0,0	0,1,0,1	1,0,0,1	1,0,0,0			0,1,0,0	0,0,0,1	
		mandible	1,1,3,2	2,1,0,1	1,0,0,0	1,0,0,0	1,0,2,0	0,0,0,9	1,0,0,0	0,0,0,0	2,0,2,2	2,0,2,1	1,0,0,0	1,0,2,1	1,0,2,0			
	63-2	maxilla				0,0,0,0	0,9,0,0	0,0,0,0	1,0,0,0				0,0,0,0	0,0,0,1	0,1,0,0		0,2,0,1	
		mandible		2,0,0,9	9,0,0,1	0,1,0,0	0,0,0,0	0,9,0,0	0,0,0,0			0,9,0,0	0,1,0,1	1,0,0,0	9,0,1,9			
	64-1	maxilla	0,1,0,0	0,1,0,0	1,1,1,1	0,0,0,1	1,1,0,0	9,9,9,1	0,0,0,0	1,0,0,0		0,1,1,1	1,2,0,0	1,2,0,2	2,2,0,3	2,1,9,2	1,1,0,1	1,0,0,0
		mandible	1,1,1,1	1,1,1,0	0,2,1,1	2,1,2,0	0,1,1,1	1,0,1,1	2,0,9,0	0,0,1,0	2,2,1,2	2,2,1,2	3,2,1,0	1,1,1,1	2,1,2,2			

Cemetery	Burial	Element	RI1	RI2	RC	RP1	RP2	RM1	RM2	RM3	LI1	LI2	LC	LP1	LP2	LM1	LM2	LM3
	65	maxilla	0,0,0,9	0,0,0,9	0,0,0,0	0,0,0,0	0,0,0,0	0,1,0,0	0,0,0,0	0,0,0,0	0,0,0,9	0,0,0,9	0,0,0,0	0,0,0,0		0,2,9,9	9,9,0,2	2,1,0,0
		mandible		0,0,0,1			1,0,9,0	0,0,0,0	9,0,0,0	1,0,1,1		0,0,0,0		1,0,9,1	1,1,9,0			
	66-1	maxilla	1,1,0,0	0,0,0,1	1,0,0,1	0,0,0,1	1,1,0,0	0,1,0,1			2,0,1,0	0,0,0,1	2,0,0,1	1,1,0,1	1,0,0,1	0,1,0,0	1,0,0,0	
		mandible	3,1,2,2	2,1,1,1	3,1,0,1	2,0,0,1	1,0,1,1	1,0,1,0		9,0,0,0	3,3,1,3	2,2,2,2	3,2,1,2	2,2,2,2	1,1,2,1			0,0,0,0
	67	maxilla	0,0,0,0					0,0,0,0								0,0,0,0		
		mandible	1,0,0,0	0,0,0,0							0,0,0,0	0,0,0,0				0,9,1,0		
	69-1	maxilla	0,1,0,0	1,0,0,1	1,0,0,0	1,0,0,1		9,1,9,1	0,0,0,0	0,0,0,0	0,0,0,0	0,1,9,0	0,1,0,0	1,0,0,1	0,0,0,0	0,1,0,1	1,0,0,9	9,0,0,9
		mandible	1,0,0,0	1,0,0,0	0,1,0,1	2,1,1,1	0,0,0,1	1,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,1,0,0	0,0,0,0	0,0,0,0	0,9,9,9			
	69-2	maxilla	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,9,0,0	0,1,0,0	9,9,0,0	0,0,0,0			0,0,0,0	9,1,0,0	0,1,0,0	1,0,0,0	1,1,0,0	0,0,0,0
		mandible	2,2,0,1	2,1,0,1	1,1,0,0	0,0,0,0	0,0,0,0						2,0,1,1	0,0,0,1	1,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0
	70	maxilla																
		mandible	0,0,0,0	0,1,0,0	0,3,1,0	0,2,0,0	0,0,9,1				0,0,0,0	0,1,1,0	0,3,1,0	1,1,2,2	2,2,2,1			
	71	maxilla				0,9,0,1		0,9,2,3		0,0,0,0								
		mandible						1,1,2,0										
	73	maxilla		0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0		0,1,0,0	1,1,0,0	1,1,0,0		1,0,0,0		0,0,0,0	0,0,0,0
		mandible	1,1,1,1	1,1,1,1	1,0,0,1	1,0,1,0	1,0,1,0	0,0,9,0	0,0,0,0	0,0,0,0	2,2,0,1	1,1,1,1		1,0,0,1	0,0,0,0			
	74	maxilla	0,0,0,1	1,1,0,0	1,0,0,0	0,0,0,1	0,1,0,1	1,1,0,1	1,1,0,0	0,0,0,0	0,0,0,0	0,1,0,0	0,0,0,0	0,0,0,0	1,0,0,0	1,0,9,0	0,0,0,0	0,0,0,0
		mandible	1,0,0,1	1,0,2,2	1,0,2,1	0,0,0,1	1,0,0,0				1,0,0,0	0,0,0,0	0,0,0,0			0,0,0,0	0,0,0,0	0,0,0,0
	75	maxilla	1,1,0,2	1,2,0,0	1,1,0,0	0,1,0,0	0,2,0,0	1,1,0,1	0,0,0,0	0,0,0,0	0,0,0,1	1,0,0,0	1,2,0,1	9,9,0,1	0,1,0,0	0,1,0,0	0,0,0,0	0,0,0,0
		mandible	0,1,0,1	1,1,0,1		1,1,1,1	0,0,0,0	0,0,1,1	0,0,0,1	0,1,0,0	1,1,0,1	1,2,0,9	1,1,0,0	1,1,0,0	1,0,1,0			
	76	maxilla				2,0,9,9								1,0,9,9				
		mandible		0,1,0,0	0,2,0,1		1,0,9,1				1,0,0,0	0,1,0,0	0,1,0,1	0,1,0,0	0,1,9,0		0,1,0,0	
	77	maxilla						0,0,0,0					1,0,0,0	0,0,0,1	1,0,0,0			
		mandible	2,2,0,2	1,1,0,1		1,2,0,1	1,0,0,0			0,0,0,0		1,1,9,3	2,0,0,0	0,0,0,1	1,0,0,0			
	78-1	maxilla						0,0,0,0	0,0,0,0					1,0,0,0				
		mandible																
	78-2	maxilla			1,0,0,1	2,1,0,2	1,2,0,1	1,9,9,2	1,1,0,0					9,0,0,1				
		mandible																
	78-3	maxilla			1,0,0,1	0,1,0,1	1,1,0,1	0,1,0,1	0,0,0,0	0,0,0,0			1,1,0,2	1,1,0,2	2,1,0,1	1,1,0,1	1,0,0,0	0,0,0,0
		mandible		1,0,0,1	1,1,0,1	1,1,0,1	1,0,1,1	0,0,0,9	9,0,0,0	0,0,0,0			0,0,0,0	1,1,2,1	2,1,1,1			

Cemetery	Burial	Element	RI1	RI2	RC	RP1	RP2	RM1	RM2	RM3	LI1	LI2	LC	LP1	LP2	LM1	LM2	LM3
Ust'-Ida I	79-1	maxilla	9,1,0,0		1,1,0,0	0,0,9,0	0,9,9,0	0,3,0,0	0,1,0,0	0,0,0,0								
		mandible																
	85	maxilla	0,1,0,1	0,0,0,0	0,0,0,0	0,1,0,0	0,0,0,0				0,0,0,0	0,0,0,0	1,0,0,0	0,0,0,0	0,0,0,0			
		mandible	0,1,9,0	0,1,0,0		1,1,1,1	1,0,0,0				2,2,1,2	0,2,0,1	1,1,0,0	1,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0
	88	maxilla						0,0,0,0								0,0,0,0		
		mandible	0,0,0,0	0,0,0,0							0,0,0,0							
	90	maxilla	0,0,0,0	0,0,0,0	0,0,0,0	9,0,0,1	1,0,0,1	0,1,0,0	0,0,0,0	0,0,0,0	0,0,0,0	1,0,0,1	1,0,0,0	1,0,0,0	0,1,0,0	1,1,0,0	1,1,0,0	0,0,0,0
		mandible	1,0,0,0	0,0,0,1	1,0,0,0	0,0,0,1	0,0,0,0	0,0,1,0	0,0,0,0	0,0,0,0	1,0,0,0	1,0,0,0	0,0,0,0	0,0,1,0	0,0,0,0			
	92	maxilla	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0							0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0		
		mandible	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0		0,0,0,0			0,0,0,1	0,0,0,0		0,0,0,0				
	93-2	maxilla	0,1,0,0	0,0,0,0	0,1,0,0	0,2,0,0	0,0,0,1	1,1,9,0	0,1,0,0	0,0,0,0	0,1,0,0	0,1,0,0	0,2,0,0	0,9,0,0	0,2,9,9	1,0,0,0	9,1,1,0	0,0,1,9
		mandible	0,0,0,0	0,0,0,0	0,1,0,0	0,1,0,1	0,2,1,0	0,0,9,9	9,9,0,0	0,0,9,9	9,0,0,0	0,0,0,0	0,1,0,0	0,1,9,0	1,1,1,1			
	96-2	maxilla		0,0,0,0	0,0,0,1	0,0,0,1	0,0,0,0	0,1,0,0	0,2,0,9				0,0,0,0	0,1,0,0	0,0,0,0	1,0,0,1	0,1,0,1	0,0,0,0
		mandible	1,1,1,0	0,0,0,0	0,1,0,1	0,0,0,1	1,0,2,0	1,0,0,0	0,0,9,9		1,0,1,0	0,0,1,0	0,0,0,0	1,0,1,0	0,0,0,1			
	104	maxilla	0,0,0,0	0,0,0,0	1,1,0,0		1,0,0,1	0,0,0,0	0,0,0,0	0,0,0,0	9,0,0,0	0,0,0,0	0,0,0,1	0,0,0,0	1,1,0,0	1,0,0,0	0,0,0,0	0,0,0,0
		mandible	0,0,9,0	9,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0				0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	9,0,0,0	0,0,0,0	0,0,0,0
	108-1	maxilla																
		mandible		1,0,0,0	0,2,1,0	0,1,1,0	0,0,1,0	1,1,0,0	0,0,1,0		0,0,0,0	0,0,0,0		0,1,0,0	0,0,2,0	0,1,1,0		
	108-3	maxilla	0,0,0,0	0,0,0,0	0,0,0,1	0,0,0,1		0,0,9,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,9	0,1,0,0	0,0,0,0	0,0,0,0	0,0,0,0
		mandible	0,0,0,0	0,0,0,0	0,0,0,0	1,0,0,1	0,0,0,1	1,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,1	0,0,0,0			
	5-1-1	maxilla		0,0,0,0				0,0,0,0			0,0,0,0	0,0,0,0				0,0,0,0		
		mandible		1,0,0,0	0,0,0,0	0,0,0,0		0,0,0,0			1,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0				
	6-1-1	maxilla				0,1,0,0	1,0,0,1					9,1,0,1	0,1,0,0	0,9,9,0	0,9,0,1	1,9,0,9	1,2,0,0	
		mandible					1,0,0,0	9,0,9,1										0,0,1,1
	8-1-1	maxilla						0,0,0,0								0,0,0,0		
		mandible	0,0,0,0								0,0,0,0					0,0,0,0		
	9-1-1	maxilla						0,0,0,0										
		mandible						0,0,0,0			0,0,0,0							
	10-1-1	maxilla	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0		0,0,0,0			0,0,0,0	0,0,0,0				0,0,0,0		
		mandible	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0					0,0,0,0	0,0,0,0				0,0,0,0		

Cemetery	Burial	Element	RI1	RI2	RC	RP1	RP2	RM1	RM2	RM3	LI1	LI2	LC	LP1	LP2	LM1	LM2	LM3
	11-1-1	maxilla	0,0,0,0	0,0,0,0	0,0,0,0	0,9,0,0	0,0,0,0	0,0,0,0	0,0,0,0		0,0,0,0		0,0,0,0	0,0,0,0		0,0,0,0		
		mandible	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0			0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0			
	12-1-1	maxilla	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0		0,1,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	9,0,0,0	0,0,9,0	0,0,0,0
		mandible			0,0,0,0							0,0,0,0		0,0,0,0		0,0,0,0		
	14-1-1	maxilla		1,1,0,0		0,0,0,0	0,0,0,0	0,1,0,1	0,0,0,0	0,0,0,0		0,0,0,0	1,0,0,0	0,0,0,0				0,0,0,0
		mandible	1,0,1,1	1,1,0,1	1,0,0,9	0,0,0,0	0,0,0,1			0,0,0,0		1,0,1,1	1,0,0,1				0,0,0,0	
	L-16-2-1	maxilla						0,0,0,1			0,0,0,0	0,0,0,0		0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	
		mandible	1,0,0,1	1,0,0,1	1,0,0,0	0,9,0,0	0,0,9,0	0,0,9,1		0,0,0,0	1,0,0,1	9,0,0,0	0,0,0,0		0,0,0,0			0,0,0,0
	L-18-1-1	maxilla	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0		0,0,0,0	0,0,0,0	0,0,0,0					
		mandible		0,0,0,0		0,0,0,0						0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0		0,0,0,0	
	L-19-1-1	maxilla				0,0,0,0	1,0,0,0							0,0,0,0	1,1,0,0			
		mandible	1,0,0,1		1,1,0,1	1,0,0,0	0,0,1,0					1,0,0,1		0,0,0,0	0,0,0,0	0,1,0,0	0,0,0,0	0,0,0,0
	20-1-1	maxilla	0,0,0,0	0,1,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0		9,0,0,0	0,0,0,0		0,0,0,0		0,0,0,0	0,0,0,0	
		mandible	0,0,0,0	0,0,1,0		0,0,1,0	0,0,0,0	0,0,0,0			0,0,0,0	0,0,0,0		0,0,0,0	0,0,1,0			
	20-2-1	maxilla			0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0		0,0,0,0	0,0,0,0	0,0,0,0		0,0,0,0	0,0,0,0	
		mandible	0,1,0,0	1,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0				1,0,0,0		0,0,0,0	0,0,0,0	0,0,0,0	9,0,0,0	0,0,0,0	0,0,0,0
	21-2-1	maxilla														0,0,0,0		
		mandible	0,0,0,0															
	22-1-1	maxilla					0,0,0,0	0,0,0,0	0,0,0,0							9,9,0,0	0,0,0,0	
		mandible	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0				0,0,0,0	0,0,0,0	0,0,0,0			
	24-1-1	maxilla		0,0,0,0				0,0,0,0	0,0,0,0							0,0,0,0	0,0,0,0	
		mandible						0,0,0,0	0,0,0,0									
	25-2-1	maxilla						0,0,0,0								0,0,0,0		
		mandible	0,0,0,1								0,0,0,0	0,0,0,0						
	25-3-1	maxilla														0,0,0,0		
		mandible														0,0,0,0		
	26-1-1	maxilla		0,0,0,0		0,0,0,0		0,0,0,0	0,0,0,0							0,0,0,0		
		mandible	1,0,0,1		0,0,0,0	0,0,0,0	0,0,0,0				0,0,0,0		0,0,0,0	0,0,0,0	0,0,0,0		0,0,0,0	
	26-4-1	maxilla									0,0,0,0	0,0,0,0	0,0,0,0			0,0,0,0		
		mandible			0,0,0,0		0,0,0,0									0,0,0,0		

Cemetery	Burial	Element	RI1	RI2	RC	RP1	RP2	RM1	RM2	RM3	LI1	LI2	LC	LP1	LP2	LM1	LM2	LM3
	26-5-1	maxilla																
		mandible						0,0,0,0								0,0,0,0		
	29-1-1	maxilla						9,0,9,9	0,0,0,0					0,0,0,0	0,0,0,0	0,9,9,0	0,0,9,0	
		mandible	0,0,0,0	1,0,0,9	9,0,0,0	0,0,0,1					9,0,0,0	9,0,0,1	1,0,0,0	0,0,0,0	0,0,0,1	0,0,9,0	0,0,0,0	0,0,0,0
	30-1-1	maxilla			0,0,0,0	0,0,0,0							0,1,0,0	0,0,0,0		0,0,0,0		
		mandible			9,0,0,1	1,0,1,0	1,1,0,1					0,0,0,0	0,0,0,1	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0
	31-1-1	maxilla			0,0,0,0	0,0,0,0		0,0,0,0	0,0,0,0		0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0		0,0,0,0	0,0,0,0	
		mandible	1,0,1,0	0,0,1,0	0,0,0,0	0,0,0,0					1,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0		0,0,0,0		
	32-1-1	maxilla		0,0,0,0		0,0,9,0												
		mandible	0,0,0,0					0,0,0,0			0,0,0,0					0,0,0,0		
	33-1-1	maxilla			0,9,0,0			0,0,0,0	0,0,0,0						1,1,0,0	1,1,0,1	0,0,0,0	
		mandible	2,1,1,1	9,0,1,1							2,0,0,1	1,0,0,1	1,0,1,1	1,0,1,1	0,0,0,0			
	33-2-1	maxilla						0,0,0,0								0,0,0,0		
		mandible																
	38-1-1	maxilla																
		mandible		1,0,1,0	9,0,9,9							2,9,1,9	0,0,0,1	1,0,0,1				
	44-1-1	maxilla	0,0,0,0					0,0,0,0								0,0,0,0		
		mandible		0,0,0,0		0,0,0,0								0,0,0,0				
	44-3-1	maxilla	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0		0,0,0,0			0,0,0,0	0,0,0,0		0,0,0,0			
		mandible	0,1,0,1	1,1,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0			0,0,0,0	0,1,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	
	45-1-1	maxilla																
		mandible	0,0,9,0	0,0,0,0			0,0,0,1	0,0,0,0		0,0,0,0		1,0,0,0			0,0,0,0		0,0,0,0	
	48-1-1	maxilla			0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0				0,0,0,0	0,0,0,1	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0
		mandible		0,1,1,1	0,0,0,0	0,0,0,0	0,0,0,0					0,0,1,0	0,0,1,0	0,0,1,0	1,0,0,0	0,0,9,0	0,0,9,0	0,0,9,0
	53-1-1	maxilla					0,0,0,0	0,0,0,0	0,0,0,0							0,0,0,0	0,0,0,0	
		mandible		1,0,0,0		0,0,0,0		0,0,0,0	0,0,0,0			1,0,1,1		0,0,0,0				
	56-1	maxilla			0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,1		0,0,0,0		0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	2,1,0,1	
		mandible	1,0,0,1	1,0,0,0	0,0,0,0	0,0,0,0					0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	1,0,0,0	0,0,9,0	0,0,9,0	
	56-2	maxilla	0,0,0,0			0,0,0,0		0,1,0,1			0,0,0,0					0,0,0,0		
		mandible			0,0,0,0						1,0,0,1	0,0,0,0	0,0,0,0					

Cemetery	Burial	Element	RI1	RI2	RC	RP1	RP2	RM1	RM2	RM3	LI1	LI2	LC	LP1	LP2	LM1	LM2	LM3
Khuzhir-Nuge XIV	10	maxilla	0,0,0,0		0,0,0,0		0,0,0,0	0,1,0,0	1,0,0,1	0,0,0,0				0,1,0,0		0,1,0,0	1,0,0,0	
		mandible		1,0,0,0		0,0,0,0	0,0,0,0	0,0,0,0	0,9,1,9	0,0,0,0	1,0,0,0		1,0,0,0	9,9,0,9	0,0,0,0			
	11	maxilla	1,0,0,1	0,0,0,0	1,0,0,0	1,1,0,1	1,3,1,1	1,1,1,1			1,0,0,1	1,0,0,0		1,1,0,1	1,1,1,1	2,2,1,1	2,1,1,1	1,1,0,1
		mandible	2,0,1,2	2,0,1,2	2,0,1,1	2,0,1,1	1,0,1,1	1,1,2,1	1,1,3,1	1,2,2,2	3,1,9,2	2,0,1,1	1,0,0,1	2,1,1,1	2,0,1,1		1,2,1,1	2,1,1,1
	12	maxilla	9,0,0,1	1,0,0,0	1,0,0,1	1,1,0,1	9,1,0,9	9,9,9,9		1,0,0,0	1,0,0,9	1,9,9,0	9,0,9,9	1,1,0,0	1,1,0,0	9,1,9,9		0,0,0,0
		mandible		9,9,9,9	1,0,9,9	9,9,9,9		0,0,1,9	1,0,1,0	0,0,0,0	0,0,0,1	9,9,9,9	1,0,0,1		0,0,0,0			
	14	maxilla	1,1,0,1	9,0,0,1	1,9,9,0	9,9,9,2	9,9,9,0	9,0,0,1	1,1,9,9		0,0,0,0		1,0,0,0		9,9,9,2	9,9,9,9		
		mandible					9,9,0,9	1,9,1,9				0,0,0,1	9,9,9,9	1,0,0,1	1,9,9,1			
	15	maxilla			0,0,0,0		0,0,0,0		0,0,0,0	0,0,0,0					1,1,0,0		1,1,0,0	1,0,0,1
		mandible				1,0,1,2	1,0,1,1	1,1,9,0	9,0,1,0	0,0,0,0			1,0,0,1		0,9,0,0			
	16	maxilla	0,0,0,0	0,0,0,0				1,0,0,0			0,0,0,0	0,0,0,0				0,1,0,0		
		mandible	0,0,0,0	0,0,0,0				1,0,0,1			0,0,0,0	0,0,0,0						
	17	maxilla						0,0,0,0								0,0,0,0		
		mandible						0,0,0,0										
	19	maxilla			0,0,0,0	0,0,9,1	1,0,0,0	0,0,9,9	1,0,0,0								0,0,0,0	
		mandible										0,0,0,0		0,0,0,0	0,0,0,0			
	27-1	maxilla	0,0,0,0	0,0,0,0	0,1,0,0	0,0,0,1	1,0,1,0	0,1,0,1	0,0,0,0			0,0,0,0		0,0,0,0	1,1,9,0	0,1,9,1	1,1,0,0	1,0,0,1
		mandible			9,9,0,0	9,9,9,0	0,0,0,0	0,0,0,0				9,9,9,9		0,0,0,0	0,0,0,9	9,0,0,0	9,9,9,9	
	27-2	maxilla	0,0,0,0	0,0,0,0				0,0,0,0				0,0,0,0						
		mandible	0,0,1,0	0,0,0,0							0,0,1,0	0,0,1,0				0,0,0,0		
	27-3	maxilla						0,0,0,0								0,0,0,0		
		mandible	0,0,0,0					0,0,0,0			0,0,0,0	0,0,0,0						
	29	maxilla																
		mandible																
	32	maxilla						9,1,9,9										
		mandible			0,0,0,0			0,0,1,0							0,0,0,0			
	34	maxilla	1,0,0,0	9,9,9,2	1,0,9,1	2,9,9,2	2,9,9,1	1,9,9,1	9,9,9,0	9,9,0,0		9,9,9,1	9,9,9,9	9,9,9,9	9,9,9,1	1,9,0,0	9,9,9,9	
		mandible		1,0,0,1	9,0,9,0	1,9,0,0	1,1,1,1	1,0,9,9	9,9,1,9	9,9,2,9		9,0,0,0	0,0,9,1	1,0,9,1	1,0,1,1			
	35-1	maxilla	0,0,0,1	0,0,0,0	0,1,0,0	0,9,0,1	0,0,0,1	9,1,9,9	0,0,9,9			0,0,0,0		0,0,0,1	0,1,0,0	1,1,9,9	0,0,0,0	
		mandible	9,1,9,1	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	9,9,0,0	0,0,0,0		2,0,1,0	1,0,0,0	0,0,0,0	0,0,0,1	0,0,0,1			



Cemetery	Burial	Element	RI1	RI2	RC	RP1	RP2	RM1	RM2	RM3	LI1	LI2	LC	LP1	LP2	LM1	LM2	LM3
	35-2	maxilla	0,0,0,0	0,0,0,0				0,0,0,0								1,2,9,1		
		mandible	2,0,1,1	0,0,0,1							2,0,1,1	1,0,1,0				0,0,1,1		
	36-1	maxilla						0,9,9,9							9,0,9,9	0,9,9,9		
		mandible												9,9,9,0	0,9,0,0			
	37-1	maxilla	9,0,0,9	9,9,0,9	0,0,0,0	0,0,0,0	0,0,0,0	0,1,0,9	0,0,0,0			9,0,9,0	0,1,0,0	1,0,0,0	0,0,0,0	0,1,0,0	0,0,0,0	
		mandible		1,1,0,1	0,9,9,0		0,0,0,0	9,9,1,0	0,0,1,0			0,1,9,2	0,0,9,0		0,0,0,0			
	37-2	maxilla	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,1,0,1	0,0,0,0				0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	
		mandible	2,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0				1,0,1,1	1,0,0,0		0,0,0,0	0,0,0,0			
	38	maxilla		0,0,9,0		0,1,9,0								9,9,9,2	9,9,0,0			
		mandible			0,0,0,0	0,9,0,9		9,9,9,9					9,9,9,0		1,9,0,0			
	39	maxilla	0,0,0,0	0,0,9,0									0,0,0,0					
		mandible		9,0,9,9				0,1,0,0										
	44	maxilla				0,9,9,9	9,0,9,1	9,0,9,0	0,9,9,9		0,9,9,0	0,9,0,1	9,9,0,0	0,0,9,0	0,0,9,9	9,9,0,0	0,9,9,0	
		mandible		9,0,9,9	9,0,9,9	9,0,9,9		9,9,9,0	9,0,9,0		9,0,9,0	9,0,9,9	1,0,9,0	1,0,9,1	1,0,0,9			
	45	maxilla	0,0,0,0	0,0,0,0														
		mandible	0,0,0,0	0,0,0,0				9,9,0,9						0,0,0,0				
	46	maxilla			0,9,0,0	1,0,0,0		0,9,9,9					0,9,9,9	0,0,0,9	0,9,9,0			0,0,0,0
		mandible	2,0,9,1	2,0,0,1	1,0,9,0		0,9,0,1	0,9,9,9			1,0,9,1	2,9,0,9	1,0,9,9	0,0,9,0	0,9,1,1			
	48	maxilla						0,0,0,0								0,0,0,0		
		mandible	0,0,0,0	9,0,9,0							0,0,0,0	9,0,0,9						
	50	maxilla				0,0,0,0	9,9,9,9	0,1,0,0	0,0,0,0					0,0,0,0	0,0,0,0		9,9,9,9	
		mandible	0,0,0,0	1,0,0,0		0,0,0,0			0,0,0,0		0,0,0,0	0,0,0,0	0,0,0,0		0,0,0,0		0,0,0,0	
	51	maxilla	0,0,0,9	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	9,0,0,0	9,0,9,0		9,0,9,1	9,0,9,0	9,0,0,0	0,0,0,0
		mandible	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	9,0,9,0	0,0,0,1	0,0,0,0			0,0,0,0	0,0,0,0	0,0,0,0			
	52	maxilla																
		mandible				1,1,1,1	2,2,9,9											
	53	maxilla		0,0,9,0	9,0,0,0		0,0,9,0	0,9,0,0	0,9,9,0							2,9,0,1		
		mandible	1,1,0,9				1,2,2,1	1,9,1,9	2,9,1,0	0,9,9,9			0,9,9,9	1,1,1,1	9,9,1,1			0,9,0,9
	55	maxilla			0,0,0,0	0,0,0,0	0,0,0,0	0,1,9,1					9,0,0,0	0,0,0,0	0,0,0,0	9,0,0,1		0,0,0,0
		mandible		9,0,9,2	9,0,9,0			9,0,9,9						0,0,0,0	1,0,0,9			

Cemetery	Burial	Element	RI1	RI2	RC	RP1	RP2	RM1	RM2	RM3	LI1	LI2	LC	LP1	LP2	LM1	LM2	LM3
	57-2	maxilla										9,0,9,0						
		mandible		0,0,0,0	0,0,9,9							9,0,0,0				9,9,9,0		
	58-1	maxilla			9,0,9,9	1,1,9,9	9,2,9,0		9,9,0,9				1,9,0,1			9,2,9,9		
		mandible				1,0,1,1	0,0,1,1	9,0,9,1	0,0,9,9									
	58-2	maxilla																
		mandible					1,9,0,0		9,0,9,1						0,9,0,0			
	59-2	maxilla		0,1,0,0		0,0,0,1	9,0,9,1				0,0,0,0	0,0,0,0		0,0,0,0		1,0,0,0		
		mandible		1,9,0,0			1,0,1,0		0,0,1,0		0,0,0,0		9,9,0,9		0,0,1,0			
	60	maxilla			9,9,0,9	0,9,0,9	1,9,9,9						9,0,0,1	1,0,9,1	0,1,9,0	9,0,0,0		
		mandible			0,0,0,0							0,0,0,0	0,0,0,0			0,0,0,0	0,0,0,0	
	64	maxilla	0,0,0,0		0,0,0,0			0,0,9,0	0,9,9,0				9,0,0,0	0,9,9,9		0,9,0,9	9,9,9,9	1,0,9,9
		mandible		0,0,0,0	0,0,0,0		1,0,0,0	1,0,0,1	9,0,0,0	0,0,0,0		0,0,0,0	0,0,0,0	0,9,0,0				
	68	maxilla	0,0,0,0		9,0,0,0	1,0,9,1	1,1,0,1	0,0,0,0			9,0,0,0	0,0,0,0	9,0,1,0	1,0,0,0		0,9,9,0		
		mandible	2,9,1,2	2,0,1,9	1,0,9,2	2,0,1,1	1,9,1,1	0,0,1,0	1,0,1,1		2,0,0,2	1,0,0,1		2,0,9,0	0,0,0,1			
	74	maxilla																
		mandible		0,0,0,0		0,0,0,0												
	77	maxilla																
		mandible	0,1,9,9	9,9,0,9	0,0,0,9	9,0,9,0	0,0,0,0	0,0,0,0	0,0,0,0		0,0,9,0	0,0,9,0		0,0,9,9	0,0,0,0			

## Color Codes:

	present, not in occlusion (development incomplete)
	present, development complete
	missing, no associated alveolar bone
	missing, antemortem loss
	missing, postmortem loss
	missing, congenital absence
	present, no measurements due to damage
	likely present but unobservable (e.g. should be in crypt)

## Calculus Scores (x,x,x,x= UA, UP, LA,LP):

0	no calculus observed
1	small amount observed (less than 1/3 of surface)
2	moderate amount observed (between 1/3 and 2/3 of surface)
3	large amount observed (more than 2/3 of surface)
9	unobservable surface
R	Right
L	Left

## Tooth abbreviations:

I1	central incisor
I2	lateral incisor
C	canine
P1	first premolar
P2	second premolar
M1	first molar
M2	second molar
M3	third molar

Table A-2 Severity Calculus Indices for Cis-Baikal Individuals, per Quadrant

Cemetery	Burial	Age (factor)	Sex	UA #	UA total	UA %present	UA index	UP #	UP total	UP %present	UP index	LA #	LA total	LA %present	LA index	LP #	LP total	LP %present	LP index
Lokomotiv	L-1-1-1	3	1	12	24	0.50	0.083	23	40	0.58	0.087	15	24	0.63	0.067	25	32	0.78	0.000
Lokomotiv	L-4-1-1	3	2	12	24	0.50	0.167	4	40	0.10	0.000	3	24	0.13	0.000	18	32	0.56	0.389
Lokomotiv	L-7-1-1	3	2	10	24	0.42	0.000	16	40	0.40	0.188	19	24	0.79	0.000	22	40	0.55	0.045
Lokomotiv	L-8-1-1	3	1	6	16	0.38	0.000	17	36	0.47	0.000	11	24	0.46	0.000	16	32	0.50	0.000
Lokomotiv	L-9-1-1	*	2	24	24	1.00	0.583	16	40	0.40	0.938	0	24	0.00	*	0	40	0.00	*
Lokomotiv	L-10-1-1	2	1	22	24	0.92	0.000	39	40	0.98	0.000	4	24	0.17	0.000	6	40	0.15	0.167
Lokomotiv	L-10-2-1	2	1	24	24	1.00	0.000	35	40	0.88	0.086	20	20	1.00	0.100	24	40	0.60	0.000
Lokomotiv	L-11-1-1	3	1	21	24	0.88	0.000	25	32	0.78	0.000	23	24	0.96	0.000	21	40	0.53	0.000
Lokomotiv	L-13-1-1	2	1	18	24	0.75	0.167	34	36	0.94	0.029	20	24	0.83	0.750	18	40	0.45	0.167
Lokomotiv	L-14-3-1	1	3	12	24	0.50	0.000	12	20	0.60	0.000	0	24	0.00	*	0	40	0.00	*
Lokomotiv	L-15-1-1	2	1	10	24	0.42	0.000	31	40	0.78	0.161	12	24	0.50	0.583	27	40	0.68	0.259
Lokomotiv	L-16-1-1	3	1	22	24	0.92	0.909	26	40	0.65	1.192	16	24	0.67	1.063	26	40	0.65	1.192
Lokomotiv	L-18-1-1	3	2	0	8	0.00	*	9	32	0.28	0.000	0	24	0.00	*	0	8	0.00	*
Lokomotiv	L-19-1-1	3	1	5	20	0.25	0.000	26	40	0.65	0.154	6	20	0.30	0.000	18	36	0.50	0.556
Lokomotiv	L-20-1-1	2	2	16	24	0.67	0.063	23	40	0.58	0.087	23	24	0.96	0.478	28	40	0.70	0.321
Lokomotiv	L-20-2-1	3	1	14	24	0.58	0.357	25	40	0.63	0.960	10	20	0.50	0.200	18	32	0.56	0.333
Lokomotiv	L-21-1-1	3	2	4	24	0.17	0.000	0	28	0.00	*	12	24	0.50	0.000	15	36	0.42	0.000
Lokomotiv	L-23-1-1	2	1	19	24	0.79	0.000	36	40	0.90	0.111	23	24	0.96	0.043	24	40	0.60	0.208
Lokomotiv	L-24-3-1	1	3	0	16	0.00	*	3	8	0.38	0.000	0	12	0.00	*	8	8	1.00	0.125
Lokomotiv	L-24-4-1	1	3	11	16	0.69	0.000	7	8	0.88	0.000	16	16	1.00	0.000	4	8	0.50	0.000
Lokomotiv	L-25-1-1	3	2	0	24	0.00	*	0	40	0.00	*	6	24	0.25	0.000	13	40	0.33	0.077
Lokomotiv	L-25-5-1	3	1	4	20	0.20	0.000	25	40	0.63	0.000	0	24	0.00	*	0	40	0.00	*
Lokomotiv	L-27-1-1	1	1	24	24	1.00	0.000	28	36	0.78	0.000	9	24	0.38	0.111	20	32	0.63	0.000
Lokomotiv	L-28-1-1	3	2	12	24	0.50	0.167	13	36	0.36	0.231	18	24	0.75	0.889	24	32	0.75	0.208
Lokomotiv	L-30-1-1	3	1	17	24	0.71	0.176	18	40	0.45	0.000	8	20	0.40	0.125	14	36	0.39	0.786
Lokomotiv	L-30-2-1	3	1	6	24	0.25	0.167	18	40	0.45	0.056	16	24	0.67	0.125	8	36	0.22	0.250

Cemetery	Burial	Age (factor)	Sex	UA #	UA total	UA %present	UA index	UP #	UP total	UP %present	UP index	LA #	LA total	LA %present	LA index	LP #	LP total	LP %present	LP index
Lokomotiv	L-31-1-1	3	3	4	8	0.50	0.000	18	40	0.45	0.167	0	24	0.00	*	0	40	0.00	*
Lokomotiv	L-31-2-1	2	1	20	24	0.83	0.350	28	40	0.70	0.321	24	24	1.00	0.667	24	40	0.60	0.208
Lokomotiv	L-33-1-1	3	1	7	16	0.44	0.143	37	40	0.93	0.189	8	24	0.33	0.375	28	40	0.70	0.321
Lokomotiv	L-34-1-1	3	2	17	20	0.85	0.000	26	40	0.65	0.115	19	24	0.79	0.000	20	40	0.50	0.000
Lokomotiv	L-35-1-1	*	3	21	24	0.88	0.524	31	40	0.78	0.613	24	24	1.00	0.958	22	40	0.55	0.818
Lokomotiv	L-37-1-1	2	2	0	24	0.00	*	0	40	0.00	*	6	24	0.25	0.167	10	32	0.31	0.100
Lokomotiv	L-38-2-1	3	2	20	24	0.83	0.300	35	40	0.88	0.229	16	24	0.67	0.438	18	40	0.45	0.500
Lokomotiv	L-39-1-1	2	2	20	24	0.83	0.050	18	36	0.50	0.000	14	24	0.58	0.143	27	40	0.68	0.000
Lokomotiv	L-41-1-1	1	2	4	24	0.17	0.000	23	32	0.72	0.000	0	24	0.00	*	19	32	0.59	0.105
Lokomotiv	L-41-2-1	1	3	0	8	0.00	*	0	8	0.00	*	4	8	0.50	0.000	8	8	1.00	0.000
Lokomotiv	L-42-1-1	3	1	13	24	0.54	0.077	23	36	0.64	0.217	22	24	0.92	0.000	21	32	0.66	0.143
Lokomotiv	L-43-2-1	2	2	16	24	0.67	0.000	31	40	0.78	0.226	8	24	0.33	0.250	23	36	0.64	0.043
Lokomotiv	R-2-1-1	3	1	22	24	0.92	0.136	29	40	0.73	0.483	18	24	0.75	0.556	21	36	0.58	0.762
Lokomotiv	R-7-1-1	3	1	21	24	0.88	0.143	24	36	0.67	0.000	6	8	0.75	0.000	17	36	0.47	0.412
Lokomotiv	R-9-1	1	3	0	8	0.00	*	6	8	0.75	0.000	4	8	0.50	0.000	4	8	0.50	0.000
Lokomotiv	R-11-1	2	2	24	24	1.00	0.583	26	40	0.65	0.500	24	24	1.00	1.125	37	40	0.93	0.351
Lokomotiv	R-12-1	1	3	13	16	0.81	0.000	8	8	1.00	0.000	16	16	1.00	0.000	8	12	0.67	0.000
Lokomotiv	R-13-1	1	3	0	0	*	*	4	8	0.50	0.000	5	8	0.63	0.000	0	8	0.00	*
Lokomotiv	R-14-1	2	1	8	24	0.33	0.250	23	40	0.58	0.348	12	24	0.50	1.333	24	40	0.60	0.958
Lokomotiv	R-15-1	2	2	16	24	0.67	0.188	24	40	0.60	0.083	20	24	0.83	0.750	36	40	0.90	0.417
Lokomotiv	R-15-2	3	2	3	20	0.15	0.000	9	28	0.32	0.111	11	24	0.46	0.000	11	24	0.46	0.000
Shamanka II	6	1	1	24	24	1.00	0.417	36	40	0.90	0.222	24	24	1.00	0.875	20	40	0.50	0.750
Shamanka II	7	2	2	24	24	1.00	0.250	40	40	1.00	0.125	24	24	1.00	0.500	24	40	0.60	0.000
Shamanka II	8-1	3	1	8	24	0.33	0.500	22	36	0.61	0.456	16	16	1.00	0.563	24	32	0.75	0.667
Shamanka II	10	2	1	0	24	0.00	*	0	40	0.00	*	0	24	0.00	*	21	40	0.53	0.381
Shamanka II	11-1	1	2	12	24	0.50	0.000	32	40	0.80	0.094	12	24	0.50	0.000	28	40	0.70	0.000
Shamanka II	11-2	2	1	20	24	0.83	1.050	24	40	0.60	0.625	0	24	0.00	*	0	40	0.00	*
Shamanka II	12	2	3	0	24	0.00	*	0	40	0.00	*	12	24	0.50	1.083	28	40	0.70	0.786

Cemetery	Burial	Age (factor)	Sex	UA #	UA total	UA %present	UA index	UP #	UP total	UP %present	UP index	LA #	LA total	LA %present	LA index	LP #	LP total	LP %present	LP index
Shamanka II	13-3	1	2	4	24	0.17	0.000	20	36	0.56	0.150	0	24	0.00	*	8	32	0.25	0.000
Shamanka II	14-1	2	1	20	24	0.83	0.550	38	40	0.95	0.211	22	24	0.92	1.136	28	40	0.70	0.500
Shamanka II	14-2	2	2	12	24	0.50	0.167	36	40	0.90	0.056	16	24	0.67	0.063	27	40	0.68	0.037
Shamanka II	15	2	1	8	12	0.67	0.875	24	40	0.60	0.917	15	24	0.63	1.067	24	40	0.60	1.667
Shamanka II	17-1	2	1	20	24	0.83	0.250	32	36	0.89	0.313	20	24	0.83	0.750	20	40	0.50	0.350
Shamanka II	19	2	1	4	24	0.17	0.500	26	40	0.65	0.692	4	24	0.17	0.500	20	40	0.50	0.500
Shamanka II	21-1	2	1	23	24	0.96	0.304	37	40	0.93	0.351	11	24	0.46	0.818	24	40	0.60	0.417
Shamanka II	21-2	2	1	20	24	0.83	0.550	34	40	0.85	0.294	21	24	0.88	1.095	26	40	0.65	0.731
Shamanka II	21-3	1	3	18	24	0.75	0.000	32	32	1.00	0.000	22	24	0.92	0.500	20	32	0.63	0.150
Shamanka II	22	2	1	8	24	0.33	0.375	32	40	0.80	0.188	20	24	0.83	0.850	16	36	0.44	0.063
Shamanka II	23-1	3	1	16	24	0.67	0.063	31	32	0.97	0.613	22	24	0.92	0.727	9	20	0.45	0.778
Shamanka II	24-1	2	1	7	24	0.29	0.429	33	40	0.83	0.818	8	20	0.40	0.375	26	40	0.65	0.818
Shamanka II	26-3	1	3	0	8	0.00	*	8	8	1.00	0.125	4	12	0.33	0.250	0	8	0.00	*
Shamanka II	29-1	2	1	24	24	1.00	0.375	32	40	0.80	0.344	22	24	0.92	0.591	24	32	0.75	0.292
Shamanka II	30	3	1	21	24	0.88	1.100	36	40	0.90	0.750	21	24	0.88	1.286	24	36	0.67	1.250
Shamanka II	32	3	1	12	16	0.75	0.333	21	32	0.66	0.714	20	24	0.83	1.050	16	24	0.67	1.438
Shamanka II	33	3	1	12	24	0.50	0.250	4	40	0.10	0.000	20	24	0.83	1.300	24	40	0.60	0.750
Shamanka II	34	3	1	24	24	1.00	0.417	32	40	0.80	0.156	20	24	0.83	0.900	26	40	0.65	0.885
Shamanka II	35-1	2	1	16	24	0.67	0.063	26	40	0.65	0.346	0	24	0.00	*	0	40	0.00	*
Shamanka II	36	2	1	16	24	0.67	0.125	29	32	0.91	0.517	24	24	1.00	1.750	24	36	0.67	1.167
Shamanka II	39	3	1	24	24	1.00	0.375	38	40	0.95	0.184	16	16	1.00	0.125	28	40	0.70	0.071
Shamanka II	41	2	1	16	24	0.67	0.500	28	40	0.70	0.107	24	24	1.00	1.167	27	40	0.68	0.333
Shamanka II	42-1	3	2	20	24	0.83	0.100	10	20	0.50	0.000	21	24	0.88	0.048	19	40	0.48	0.053
Shamanka II	44-1	3	1	20	24	0.83	0.700	8	8	1.00	1.000	0	0	*	*	0	0	*	*
Shamanka II	45	2	1	16	24	0.67	0.000	27	36	0.75	0.222	24	24	1.00	0.000	19	36	0.53	0.105
Shamanka II	46	2	1	7	8	0.88	0.714	17	40	0.43	1.000	21	24	0.88	1.905	20	28	0.71	1.550
Shamanka II	47	2	2	23	24	0.96	0.217	35	40	0.88	0.371	24	24	1.00	1.333	28	40	0.70	0.429

Cemetery	Burial	Age (factor)	Sex	UA #	UA total	UA %present	UA index	UP #	UP total	UP %present	UP index	LA #	LA total	LA %present	LA index	LP #	LP total	LP %present	LP index
Shamanka II	48-1	3	1	16	24	0.67	0.063	34	36	0.94	0.147	19	24	0.79	0.105	19	36	0.53	0.105
Shamanka II	49-1	1	1	20	24	0.83	0.100	38	40	0.95	0.184	20	24	0.83	0.400	24	40	0.60	0.208
Shamanka II	50-1	2	1	24	24	1.00	1.000	35	40	0.88	0.343	24	24	1.00	2.000	26	40	0.65	0.808
Shamanka II	50-2	2	1	8	24	0.33	0.250	28	40	0.70	0.250	24	24	1.00	0.875	20	32	0.63	0.250
Shamanka II	51	2	1	20	24	0.83	0.400	31	32	0.97	0.387	23	24	0.96	0.783	24	32	0.75	0.292
Shamanka II	53-1	2	1	12	24	0.50	0.000	40	40	1.00	0.025	24	24	1.00	0.000	28	40	0.70	0.000
Shamanka II	54-1	1	2	24	24	1.00	0.167	36	40	0.90	0.056	16	24	0.67	0.438	24	32	0.75	0.042
Shamanka II	55-1	3	1	8	24	0.33	0.750	8	40	0.20	1.000	17	20	0.85	1.471	24	32	0.75	1.375
Shamanka II	55-2	1	3	0	8	0.00	*	4	8	0.50	0.000	0	4	0.00	*	4	8	0.50	0.000
Shamanka II	56-2	1	3	12	16	0.75	0.000	4	8	0.50	0.000	0	16	0.00	*	0	8	0.00	*
Shamanka II	57-1	2	2	0	24	0.00	*	0	40	0.00	*	0	24	0.00	*	16	36	0.44	0.438
Shamanka II	57-2	2	2	22	24	0.92	0.409	34	36	0.94	0.176	24	24	1.00	1.000	28	40	0.70	0.357
Shamanka II	58-1	3	1	18	20	0.90	0.333	24	36	0.67	1.000	16	16	1.00	0.625	14	16	0.88	1.000
Shamanka II	59-1	3	1	16	24	0.67	1.000	24	40	0.60	0.625	12	24	0.50	0.333	8	40	0.20	0.750
Shamanka II	59-2	1	2	24	24	1.00	0.000	12	40	0.30	0.167	8	24	0.33	0.125	0	40	0.00	*
Shamanka II	60-2	3	2	0	24	0.00	*	0	40	0.00	*	19	20	0.95	0.684	28	40	0.70	0.607
Shamanka II	61-1	2	2	24	24	1.00	0.000	31	32	0.97	0.065	20	24	0.83	0.100	16	24	0.67	0.000
Shamanka II	61-2	3	1	12	24	0.50	0.333	27	36	0.75	0.481	15	24	0.63	0.667	18	32	0.56	0.611
Shamanka II	62-5	3	1	20	20	1.00	0.050	23	40	0.58	0.043	16	24	0.67	0.000	19	32	0.59	0.053
Shamanka II	63-1	2	1	24	24	1.00	0.625	24	36	0.67	0.250	24	24	1.00	1.000	27	40	0.68	0.444
Shamanka II	63-2	2	1	8	16	0.50	0.000	27	28	0.96	0.222	13	24	0.54	0.385	21	36	0.58	0.143
Shamanka II	64-1	2	1	20	24	0.83	0.600	36	40	0.90	0.722	24	24	1.00	1.292	27	40	0.68	0.926
Shamanka II	65	3	1	20	24	0.83	0.000	32	36	0.89	0.250	8	16	0.50	0.125	20	40	0.50	0.400
Shamanka II	66-1	2	2	24	24	1.00	0.500	28	40	0.70	0.429	24	24	1.00	1.833	27	40	0.68	0.778
Shamanka II	67	1	3	4	8	0.50	0.000	8	8	1.00	0.000	16	16	1.00	0.063	3	8	0.38	0.333
Shamanka II	69-1	2	2	23	24	0.96	0.261	31	40	0.78	0.290	24	24	1.00	0.208	25	40	0.63	0.280
Shamanka II	69-2	2	2	19	24	0.79	0.053	37	40	0.93	0.135	20	24	0.83	0.800	28	40	0.70	0.036

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Shamanka II	70	3	1	0	0	*	*	0	8	0.00	*	24	24	1.00	0.458	15	20	0.75	1.067
Shamanka II	71	3	1	0	24	0.00	*	10	40	0.25	0.600	0	24	0.00	*	4	36	0.11	1.000
Shamanka II	73	1	2	20	24	0.83	0.250	28	36	0.78	0.036	20	24	0.83	0.950	27	40	0.68	0.222
Shamanka II	74	1	1	24	24	1.00	0.208	39	40	0.98	0.256	24	24	1.00	0.500	20	40	0.50	0.100
Shamanka II	75	2	1	24	24	1.00	0.625	38	40	0.95	0.237	19	24	0.79	0.684	28	40	0.70	0.429
Shamanka II	76	3	1	0	0	*	*	4	8	0.50	0.750	20	24	0.83	0.400	14	24	0.58	0.357
Shamanka II	77	2	2	8	20	0.40	0.250	8	24	0.33	0.125	15	20	0.75	1.067	20	32	0.63	0.350
Shamanka II	78-1	1	2	0	24	0.00	*	12	36	0.33	0.083	0	24	0.00	*	0	40	0.00	*
Shamanka II	78-2	2	2	4	20	0.20	0.500	17	40	0.43	0.882	0	24	0.00	*	0	40	0.00	*
Shamanka II	78-3	2	1	8	24	0.33	0.750	40	40	1.00	0.475	12	24	0.50	0.417	26	40	0.65	0.615
Shamanka II	79-1	3	2	7	24	0.29	0.429	17	40	0.43	0.235	0	24	0.00	*	0	40	0.00	*
Shamanka II	85	2	1	24	24	1.00	0.125	16	40	0.40	0.063	19	20	0.95	0.737	28	40	0.70	0.214
Shamanka II	88	1	3	0	0	*	*	8	8	1.00	0.000	12	16	0.75	0.000	0	8	0.00	*
Shamanka II	90	1	1	24	24	1.00	0.125	39	40	0.98	0.256	24	24	1.00	0.208	28	40	0.70	0.143
Shamanka II	92	1	3	20	24	0.83	0.000	12	16	0.75	0.000	18	24	0.75	0.056	12	16	0.75	0.000
Shamanka II	93-2	3	2	24	24	1.00	0.250	34	40	0.85	0.353	23	24	0.96	0.087	21	40	0.53	0.476
Shamanka II	96-2	2	2	16	24	0.67	0.125	35	40	0.88	0.229	24	24	1.00	0.333	22	32	0.69	0.364
Shamanka II	104	2	2	23	24	0.96	0.130	36	40	0.90	0.139	22	24	0.92	0.000	27	40	0.68	0.000
Shamanka II	108-1	3	1	0	24	0.00	*	0	40	0.00	*	16	24	0.67	0.250	28	36	0.78	0.393
Shamanka II	108-3	2	1	24	24	1.00	0.042	34	40	0.85	0.059	24	24	1.00	0.000	28	40	0.70	0.179
Ust'-Ida I	5-1-1	1	3	12	16	0.75	0.000	8	8	1.00	0.000	20	24	0.83	0.100	12	16	0.75	0.000
Ust'-Ida I	6-1-1	3	1	7	20	0.35	0.429	19	32	0.59	0.421	0	24	0.00	*	10	28	0.36	0.400
Ust'-Ida I	8-1-1	1	3	0	8	0.00	*	8	8	1.00	0.000	8	8	1.00	0.000	4	8	0.50	0.000
Ust'-Ida I	9-1-1	1	3	0	4	0.00	*	4	8	0.50	0.000	4	8	0.50	0.000	4	8	0.50	0.000
Ust'-Ida I	10-1-1	1	3	20	20	1.00	0.000	12	12	1.00	0.000	16	24	0.67	0.000	8	12	0.67	0.000
Ust'-Ida I	11-1-1	3	2	20	24	0.83	0.000	23	28	0.82	0.000	24	24	1.00	0.000	19	28	0.68	0.000
Ust'-Ida I	12-1-1	3	1	24	24	1.00	0.042	34	36	0.94	0.000	8	24	0.33	0.000	8	32	0.25	0.000
Ust'-Ida I	14-1-1	1	1	12	24	0.50	0.250	28	40	0.70	0.071	19	24	0.79	0.632	16	40	0.40	0.063

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Ust'-Ida I	16-2-1	2	1	8	24	0.33	0.000	20	40	0.50	0.050	23	24	0.96	0.304	21	40	0.53	0.048
Ust'-Ida I	18-1-1	1	3	24	24	1.00	0.000	16	36	0.44	0.000	12	24	0.50	0.000	16	28	0.57	0.000
Ust'-Ida I	19-1-1	2	1	0	24	0.00	*	16	32	0.50	0.188	12	24	0.50	0.583	28	40	0.70	0.107
Ust'-Ida I	20-1-1	2	1	19	24	0.79	0.053	28	32	0.88	0.000	16	24	0.67	0.063	20	36	0.56	0.100
Ust'-Ida I	20-2-1	2	2	12	24	0.50	0.000	32	40	0.80	0.000	20	24	0.83	0.150	27	40	0.68	0.000
Ust'-Ida I	21-2-1	1	3	0	8	0.00	*	4	8	0.50	0.000	4	8	0.50	0.000	0	8	0.00	*
Ust'-Ida I	22-1-1	1	2	0	24	0.00	*	18	40	0.45	0.000	16	24	0.67	0.000	24	36	0.67	0.000
Ust'-Ida I	24-1-1	1	2	4	24	0.17	0.000	16	32	0.50	0.000	0	24	0.00	*	8	32	0.25	0.000
Ust'-Ida I	25-2-1	1	3	0	16	0.00	*	8	8	1.00	0.000	12	16	0.75	0.083	0	8	0.00	*
Ust'-Ida I	25-3-1	1	3	0	16	0.00	*	4	8	0.50	0.000	0	16	0.00	*	4	8	0.50	0.000
Ust'-Ida I	26-1-1	1	3	4	24	0.17	0.000	16	32	0.50	0.000	16	24	0.67	0.125	20	32	0.63	0.000
Ust'-Ida I	26-4-1	1	3	12	24	0.50	0.000	4	32	0.13	0.000	4	24	0.17	0.000	8	32	0.25	0.000
Ust'-Ida I	26-5-1	1	3	0	0	*	*	0	8	0.00	*	0	0	*	*	8	8	1.00	0.000
Ust'-Ida I	29-1-1	3	1	0	24	0.00	*	18	40	0.45	0.000	20	24	0.83	0.150	23	36	0.64	0.087
Ust'-Ida I	30-1-1	3	2	8	24	0.33	0.125	12	28	0.43	0.000	11	24	0.46	0.182	28	40	0.70	0.179
Ust'-Ida I	31-1-1	1	3	16	24	0.67	0.000	24	24	1.00	0.000	24	24	1.00	0.167	12	20	0.60	0.000
Ust'-Ida I	32-1-1	1	3	4	16	0.25	0.000	3	16	0.19	0.000	8	16	0.50	0.000	8	8	1.00	0.000
Ust'-Ida I	33-1-1	1	3	3	24	0.13	0.000	20	32	0.63	0.250	19	24	0.79	0.789	8	32	0.25	0.375
Ust'-Ida I	38-1-1	3	1	0	0	*	*	0	20	0.00	*	11	20	0.55	0.545	4	16	0.25	0.500
Ust'-Ida I	44-1-1	1	3	4	20	0.20	0.000	8	20	0.40	0.000	4	24	0.17	0.000	8	20	0.40	0.000
Ust'-Ida I	44-3-1	1	3	20	24	0.83	0.000	16	32	0.50	0.000	24	24	1.00	0.208	28	32	0.88	0.000
Ust'-Ida I	45-1-1	2	1	4	24	0.17	0.000	4	40	0.10	0.000	11	24	0.46	0.091	20	36	0.56	0.050
Ust'-Ida I	48-1-1	2	1	8	16	0.50	0.000	36	36	1.00	0.028	16	16	1.00	0.313	25	40	0.63	0.080
Ust'-Ida I	53-1-1	1	3	0	24	0.00	*	20	32	0.63	0.000	8	24	0.33	0.500	16	32	0.50	0.000
Ust'-Ida I	56-1	3	1	12	20	0.60	0.000	32	32	1.00	0.000	24	24	1.00	0.125	18	32	0.56	0.056
Ust'-Ida I	56-2	1	3	8	24	0.33	0.000	12	20	0.60	0.167	16	24	0.67	0.125	0	16	0.00	*
Nuge XIV	10	2	3	8	24	0.33	0.000	28	40	0.70	0.214	12	24	0.50	0.250	23	40	0.58	0.043
Nuge XIV	11	3	1	20	24	0.83	0.300	32	40	0.80	1.031	23	24	0.96	1.130	36	40	0.90	1.222
Nuge XIV	12	2	3	17	24	0.71	0.353	23	40	0.58	0.435	10	24	0.42	0.400	15	40	0.38	0.200



Cemetery	Burial	Age (factor)	Sex	UA #	UA total	UA %present	UA index	UP #	UP total	UP %present	UP index	LA #	LA total	LA %present	LA index	LP #	LP total	LP %present	LP index
Khuzhir-Nuge XIV	14	3	1	17	24	0.71	0.353	8	40	0.20	0.875	4	32	0.13	0.250	9	24	0.38	0.667
Khuzhir-Nuge XIV	15	2	1	4	24	0.17	0.000	24	40	0.60	0.250	4	24	0.17	0.500	21	40	0.53	0.476
Khuzhir-Nuge XIV	16	1	3	16	16	1.00	0.000	8	8	1.00	0.250	16	16	1.00	0.000	4	8	0.50	0.500
Khuzhir-Nuge XIV	17	1	3	0	0	*	*	8	8	1.00	0.000	0	8	0.00	*	4	8	0.50	0.000
Khuzhir-Nuge XIV	19	3	2	4	20	0.20	0.000	17	40	0.43	0.176	4	24	0.17	0.000	8	40	0.20	0.000
Khuzhir-Nuge XIV	27-1	3	1	16	24	0.67	0.063	34	40	0.85	0.382	2	20	0.10	0.000	19	36	0.53	0.000
Khuzhir-Nuge XIV	27-2	1	3	12	16	0.75	0.000	4	8	0.50	0.000	16	16	1.00	0.188	4	8	0.50	0.000
Khuzhir-Nuge XIV	27-3	1	3	0	0	*	*	8	8	1.00	0.000	12	12	1.00	0.000	4	8	0.50	0.000
Khuzhir-Nuge XIV	32	3	2	0	16	0.00	*	1	32	0.03	1.000	4	24	0.17	0.000	8	24	0.33	0.125
Khuzhir-Nuge XIV	34	2	1	9	24	0.38	0.667	13	40	0.33	0.846	13	24	0.54	0.308	18	40	0.45	0.778
Khuzhir-Nuge XIV	35-1	1	1	16	24	0.67	0.125	24	32	0.75	0.292	22	24	0.92	0.273	22	32	0.69	0.091
Khuzhir-Nuge XIV	35.2	1	3	8	16	0.50	0.000	7	8	0.88	0.571	16	16	1.00	0.688	4	8	0.50	0.500
Khuzhir-Nuge XIV	36-1	3	3	0	24	0.00	*	3	40	0.08	0.000	0	12	0.00	*	4	40	0.10	0.000
Khuzhir-Nuge XIV	37-1	1	3	13	24	0.54	0.077	31	32	0.97	0.097	12	24	0.50	0.500	14	36	0.39	0.143
Khuzhir-Nuge XIV	37-2	1	3	20	24	0.83	0.000	32	40	0.80	0.063	20	24	0.83	0.300	16	32	0.50	0.000
Khuzhir-Nuge XIV	38	3	1	3	24	0.13	0.000	9	40	0.23	0.444	5	24	0.21	0.000	5	40	0.13	0.200
Khuzhir-Nuge XIV	39	1	3	11	16	0.69	0.000	0	8	0.00	*	5	16	0.31	0.000	4	8	0.50	0.250
Khuzhir-Nuge XIV	44	3	1	7	24	0.29	0.143	15	36	0.42	0.067	8	24	0.33	0.125	10	32	0.31	0.300
Khuzhir-Nuge XIV	45	1	3	12	16	0.75	0.000	0	8	0.00	*	8	16	0.50	0.000	5	12	0.42	0.000
Khuzhir-Nuge XIV	46	2	1	4	24	0.17	0.000	14	40	0.35	0.071	17	24	0.71	0.706	10	36	0.28	0.300
Khuzhir-Nuge XIV	48	1	3	4	8	0.50	0.000	8	8	1.00	0.000	12	16	0.75	0.000	0	8	0.00	*
Khuzhir-Nuge XIV	50	1	3	0	24	0.00	*	20	36	0.56	0.050	20	24	0.83	0.050	16	40	0.40	0.000
Khuzhir-Nuge XIV	51	1	1	20	24	0.83	0.000	31	40	0.78	0.032	20	24	0.83	0.000	26	40	0.65	0.038
Khuzhir-Nuge XIV	53	3	1	6	16	0.38	0.000	11	36	0.31	0.273	4	24	0.17	0.500	18	40	0.45	0.944
Khuzhir-Nuge XIV	55	3	1	7	8	0.88	0.000	26	40	0.65	0.115	4	24	0.17	0.500	8	20	0.40	0.125
Khuzhir-Nuge XIV	57.2	3	1	2	24	0.08	0.000	0	40	0.00	*	9	20	0.45	0.000	1	40	0.03	0.000
Khuzhir-Nuge XIV	58-1	2	3	4	24	0.17	0.500	6	32	0.19	1.000	0	24	0.00	*	12	32	0.38	0.500

Cemetery	Burial	Age (factor)	Sex	UA #	UA total	UA %present	UA index	UP #	UP total	UP %present	UP index	LA #	LA total	LA %present	LA index	LP #	LP total	LP %present	LP index
Khuzhir- Nuge XIV	58-2	3	1	0	24	0.00	*	0	40	0.00	*	0	24	0.00	*	8	32	0.25	0.250
Khuzhir- Nuge XIV	59-2	1	1	12	24	0.50	0.083	14	40	0.35	0.214	8	24	0.33	0.125	12	36	0.33	0.333
Khuzhir- Nuge XIV	60	3	2	4	20	0.20	0.250	12	40	0.30	0.333	12	20	0.60	0.000	8	20	0.40	0.000
Khuzhir- Nuge XIV	64	2	1	11	24	0.46	0.000	10	40	0.25	0.100	16	24	0.67	0.000	18	40	0.45	0.167
Khuzhir- Nuge XIV	68	2	1	17	24	0.71	0.059	17	20	0.85	0.353	17	24	0.71	1.000	22	32	0.69	0.636
Khuzhir- Nuge XIV	77	1	3	0	24	0.00	*	0	32	0.00	*	12	24	0.50	0.083	20	32	0.63	0.000

#### Abbreviations:

UA	Upper Anterior Quadrant	total	Total number of surface present at time of death
UP	Upper Posterior Quadrant	% present	#/total
LA	Lower Anterior Quadrant	index	Sum of calculus scores per quadrant/#
LP	Lower Posterior Quadrant		Probable
#	Number of observable surfaces		

#### Age:

1	less than 20 years
2	20-35 years
3	greater than 35 years

#### Sex:

1	Male
2	Female
3	Undetermined

## **Appendix B: Data Exploration for Statistical Analysis**

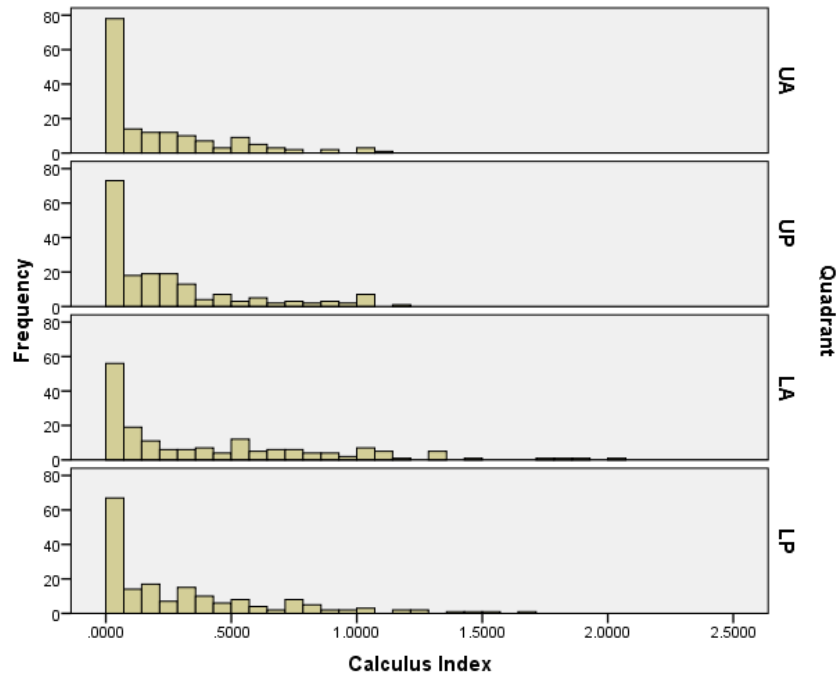


Figure B-1 Histograms showing the frequency of calculus indices within each of the four quadrants of the mouth from all individuals assessed. Data are skewed to the left, suggesting non-normality. UA= upper anterior; UP=upper posterior; LA= lower anterior; LP= lower posterior (same abbreviations apply to all graphs below).

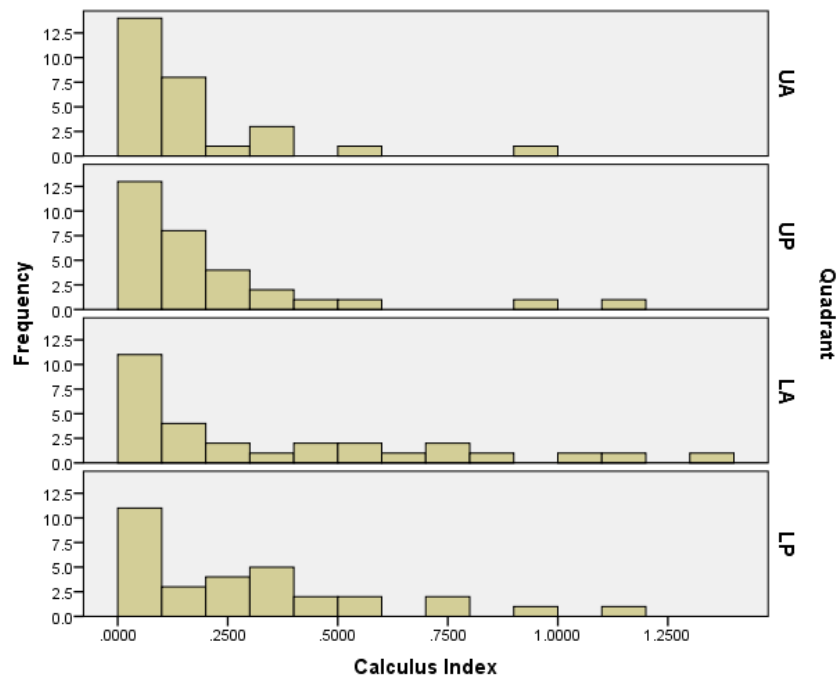


Figure B-2 Histogram showing the frequency of calculus indices within each of the four quadrants of the mouth, from Lokomotiv adults only. Data are skewed to the left, indicating non-normality.

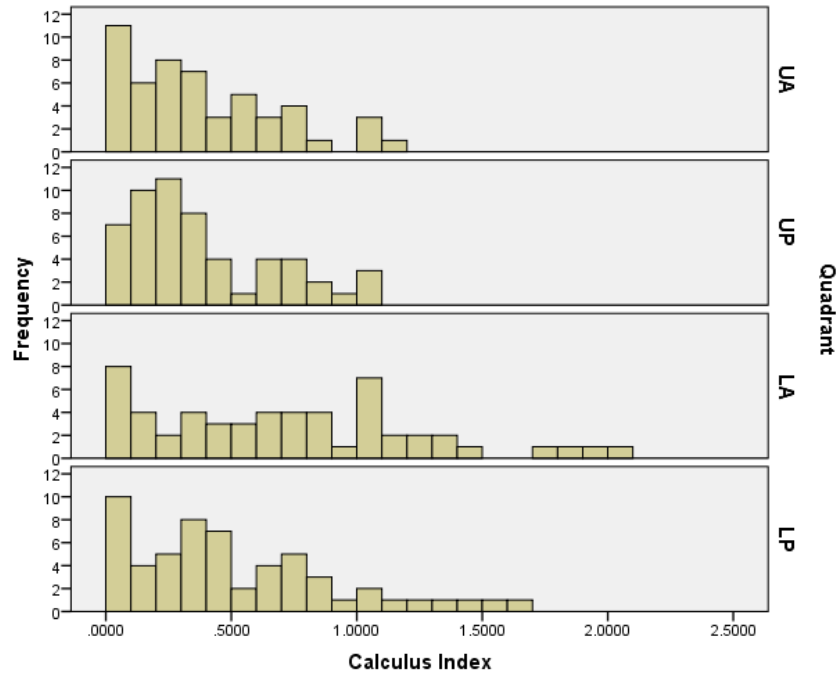


Figure B-3 Histogram showing the frequency of calculus indices within each of the four quadrants of the mouth, from Shamanka II adults only. Data are skewed to the left, indicating non-normality.

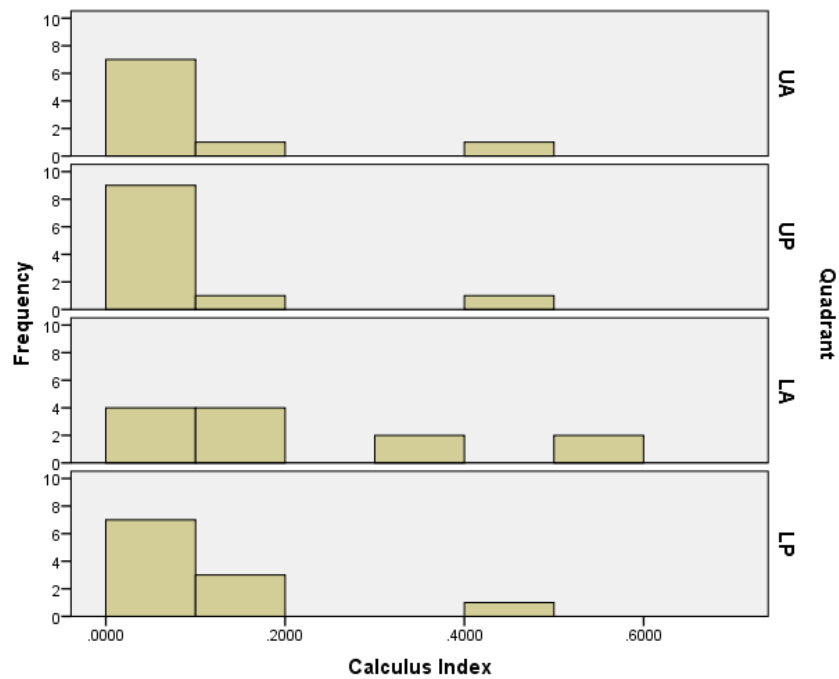


Figure B-4 Histogram showing the frequency of calculus indices within each of the four quadrants of the mouth, from Ust'-Ida I adults only. Data are skewed to the left, indicating non-normality.

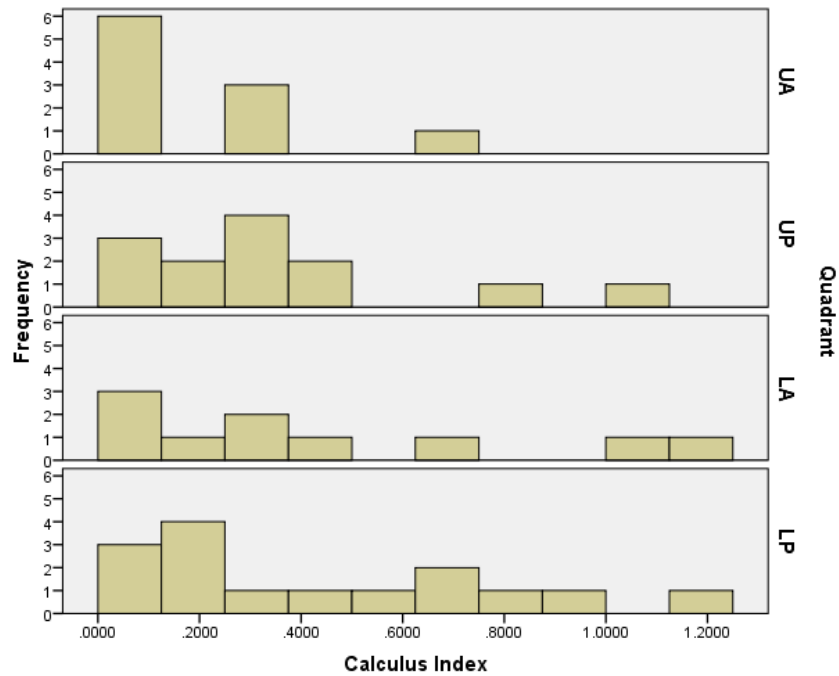


Figure B-5 Histogram showing the frequency of calculus indices within each of the four quadrants of the mouth, from Khuzhir-Nuge XIV adults only. Data are generally skewed slightly to the left, indicating non-normality.

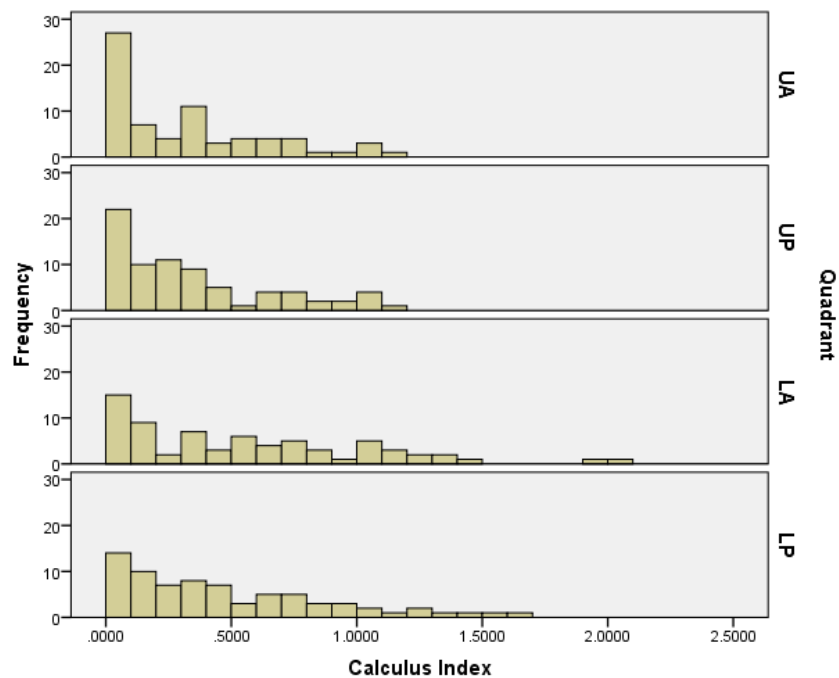


Figure B-6 Histogram showing the frequency of calculus indices within each of the four quadrants of the mouth, from all adult males included in analyses. Data are skewed to the left, indicating non-normality.

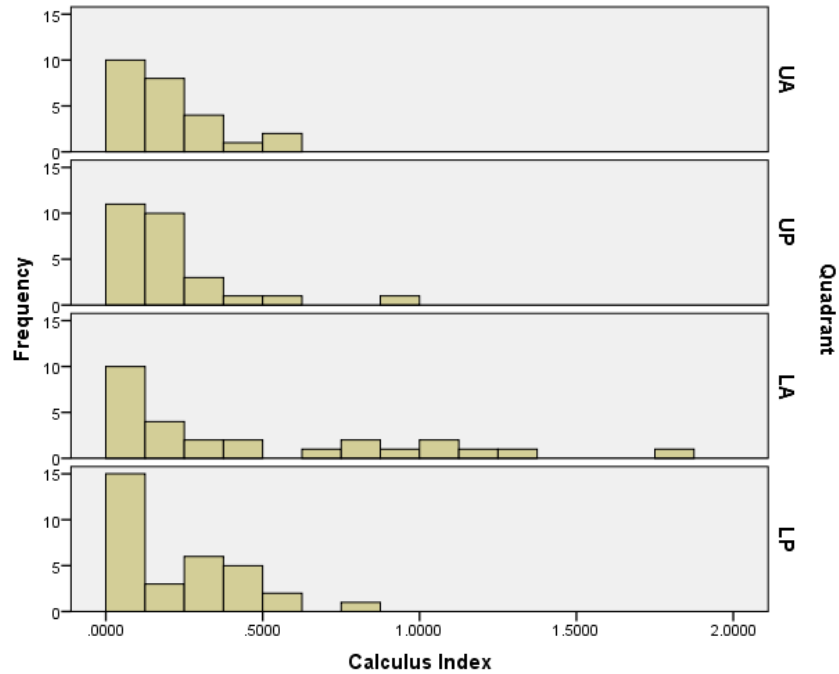


Figure B-7 Histogram showing the frequency of calculus indices within each of the four quadrants of the mouth, from all adult females included in analyses. Data are skewed to the left, indicating non-normality.

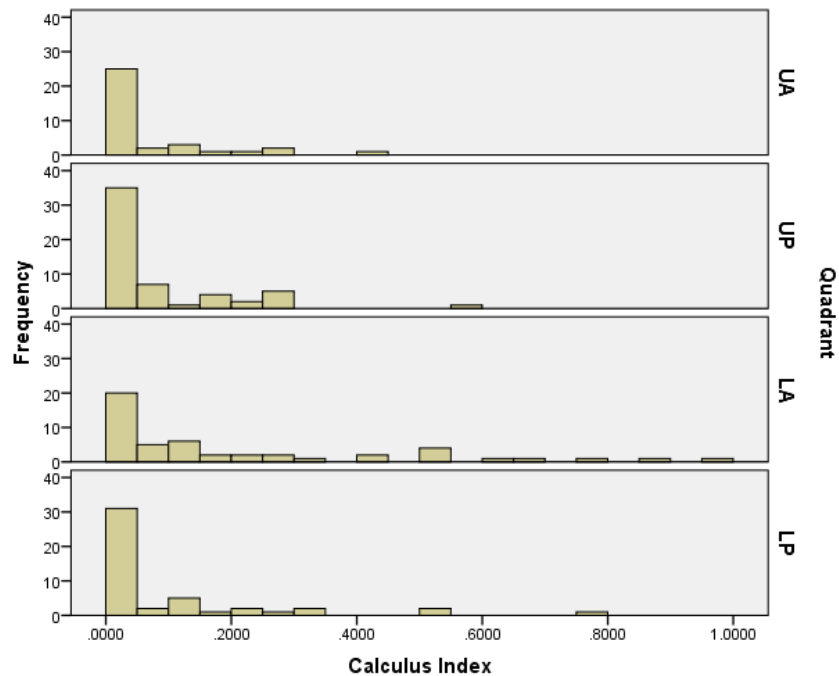


Figure B-8 Histogram showing the frequency of calculus indices within each of the four quadrants of the mouth, from all juveniles included in analyses. Data are skewed heavily to the left, indicating non-normality.

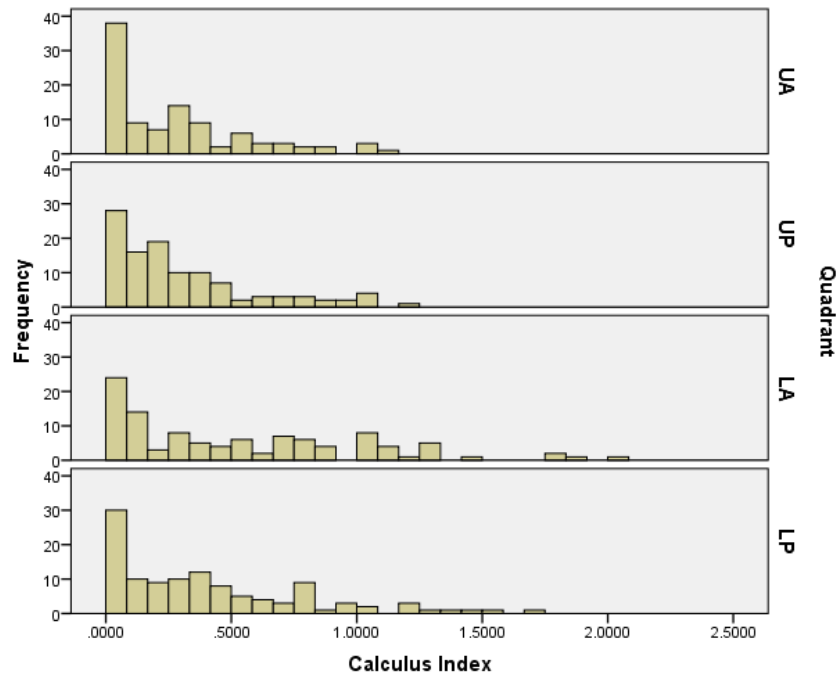


Figure B-9 Histogram showing the frequency of calculus indices within each of the four quadrants of the mouth, from all adults included in analyses. Data are skewed to the left, indicating non-normality.

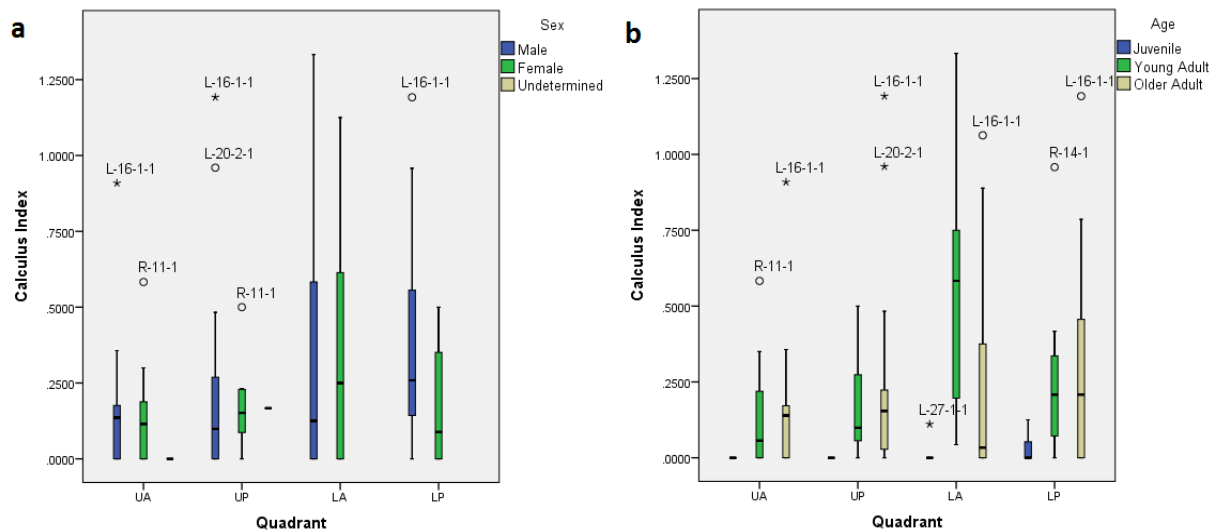


Figure B-10 Boxplots of data distributions for Lokomotiv for each oral quadrant: (a) Data separated by sex (adults only); (b) Data separated by age. Note that outliers are present within the dataset. Those represented with a circle are “out values” while those represented by stars are “far out values” (same abbreviations apply to all graphs below). Large heterogeneity of variance is present between juveniles and adult categories.



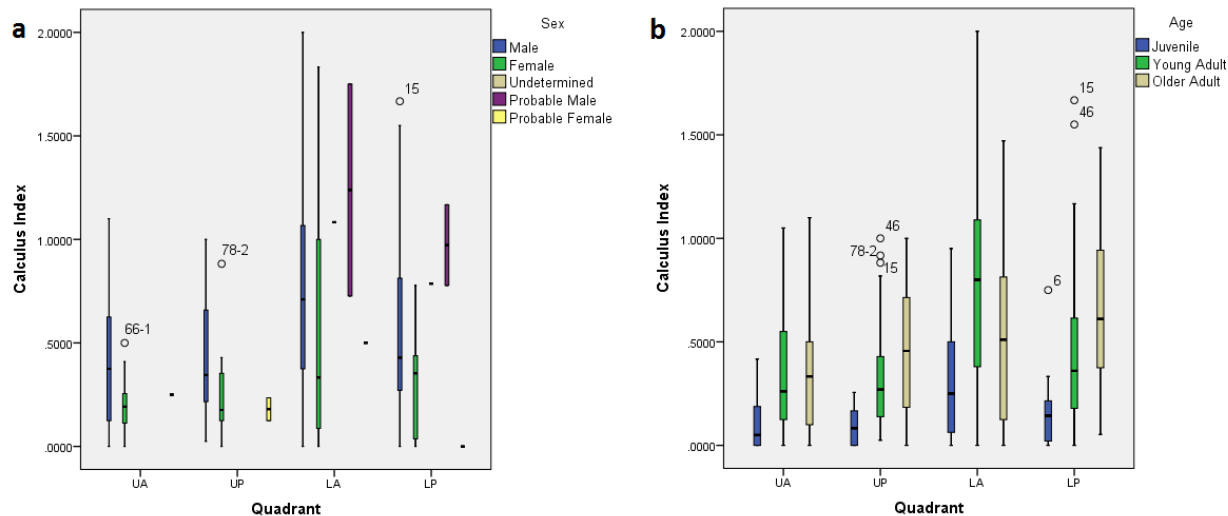


Figure B-11 Boxplots of data distributions for Shamanka II for each oral quadrant: a) Data separated by sex (adults only); (b) Data separated by age. Note that outliers are present within the dataset but none are considered “far out values”. Heterogeneity of variance is present among sex categories and between juveniles and adult categories.

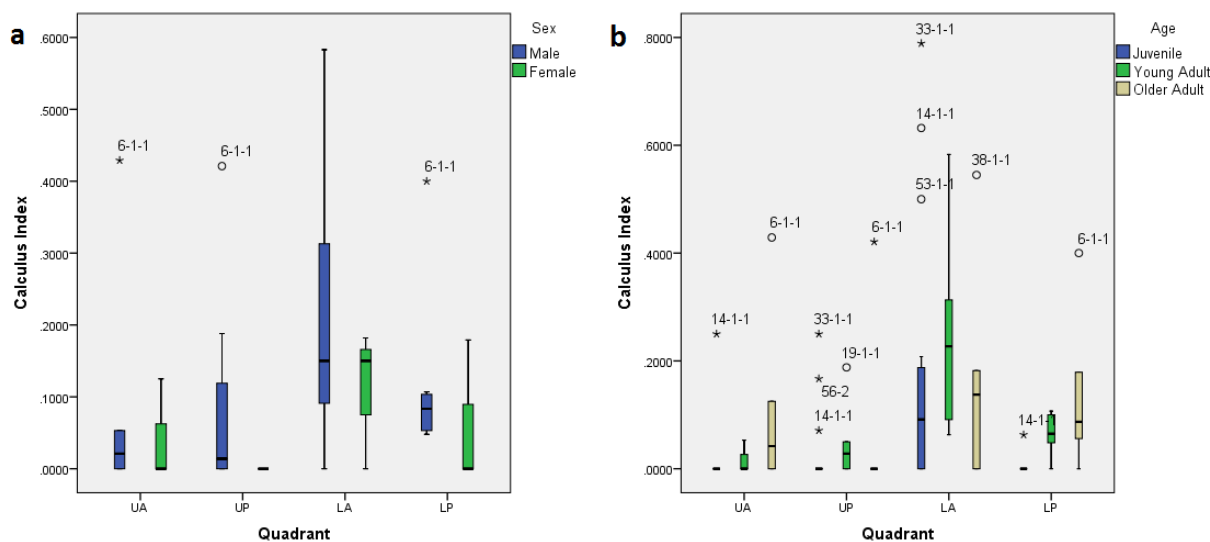


Figure B-12 Boxplots of data distributions for Ust’-Ida I for each oral quadrant: a) Data separated by sex (adults only); (b) Data separated by age. Note that outliers are present within the dataset. Heterogeneity of variance is present between male and female categories as well as among age categories.

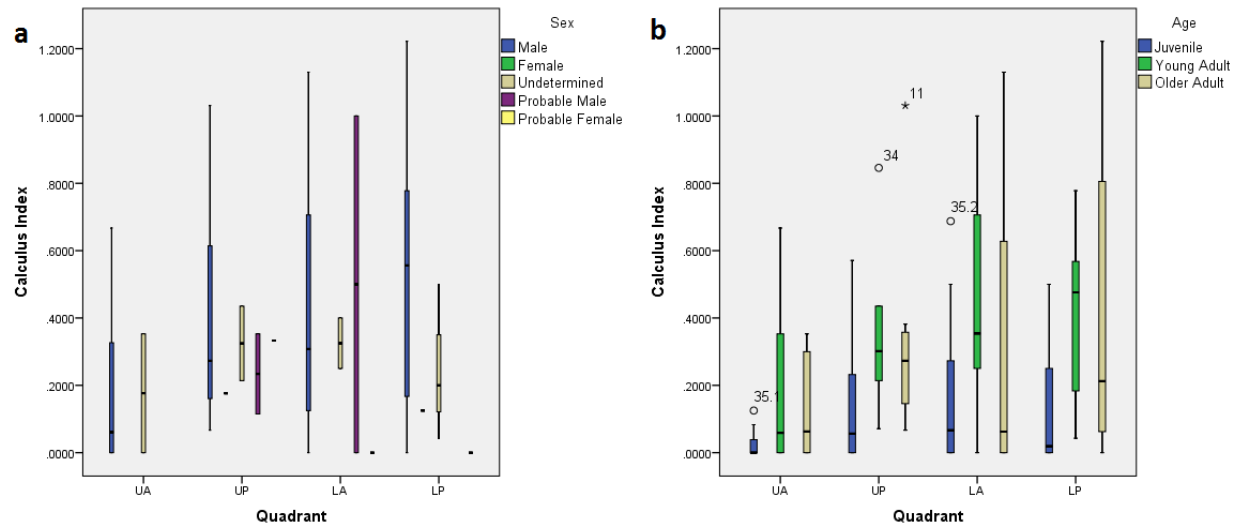


Figure B-13 Boxplots of data distributions for Khuzhir-Nuge XIV for each oral quadrant: a) Data separated by sex (adults only); (b) Data separated by age. Note that outliers are present within the dataset, but only one value is considered “far out”. Heterogeneity of variance is present between sex and age categories.

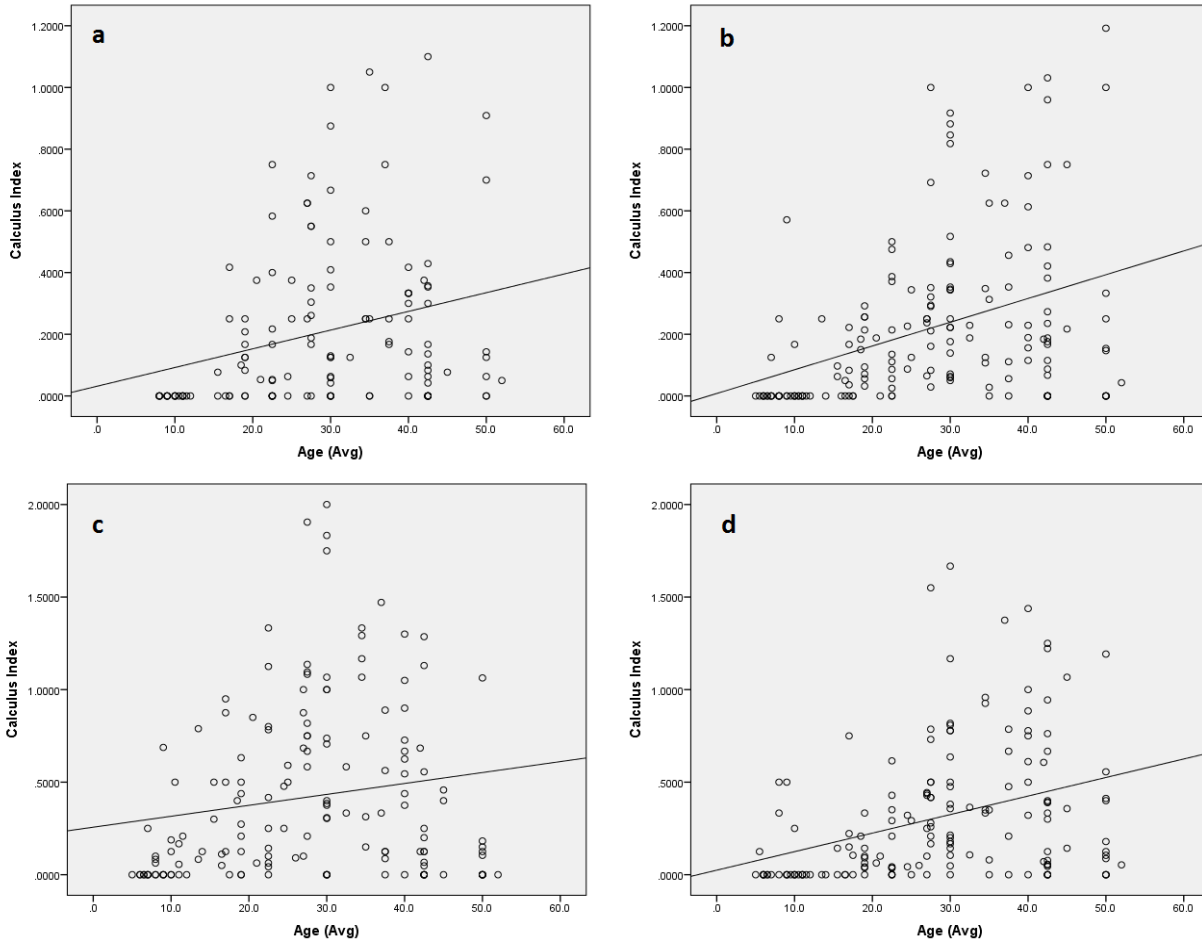


Figure B-14 Scatterplots of the data, for all individuals. A line of fit is added to show a direct relationship exists between age and dental calculus severity: (a) Upper anterior quadrant; (b) Upper posterior quadrant; (c) Lower anterior quadrant; (d) Lower posterior quadrant.

**Conclusion:** Based on the results of the data exploration, data sets for each quadrant are not normally distributed (Figures B-1 to B-9) and often display heterogeneity of variance (Figures B-10 to B-13). Therefore the assumptions of parametric testing could not be met and could not be used for this analysis. Non-parametric testing is quite common in other research projects looking at the quantity/severity of dental calculus (e.g., Greene et al. 2005; Delgado-Darius et al. 2006; Keenleyside 2008; Masotti et al. 2012) and is more flexible against non-normality and heterogeneity. For these reasons Mann-Whitney U tests were used in analyses when only two groups are being compared, while Kruskal-Wallis testing was used when analyzing differences

among three or more groups. Post hoc testing was conducted using stepwise step-down multiple correlation.

Figures B-10 to B-13 show that there are outliers present within this data set. For each outlier, the original data was rechecked and compared against pictures taken of each individual, to assess if the original score was correctly entered and accurate or whether the score was a result of a low representation of the quadrant (e.g., if the percent of surfaces present was around 30 percent). Some individuals may have scores slightly affected by poorer representation, but overall, many of the outliers were individuals with quite good representation. Therefore, the outliers were retained within the data set for the purposes of this study. There were no reasonable grounds for their removal and, based on the knowledge that individual variation is one of the main factors that can effect dental calculus, these outliers may just represent heavy calculus formers compared with the rest of the population.

Also, by plotting calculus severity for each quadrant versus age estimates, made by computing the midpoint of the age range given for each individual, it can be seen that direct relationships are evident within this data set to varying extents (Figure B-14). Due to this trend, juveniles and adults were analyzed separately since a juvenile is quite likely to have less calculus than an adult and grouping all ages together had the potential to mask the effects of other variables.

## **Appendix C: Calculus Sampling Notes for Microscopic Analysis**

Table C-1 Notes Taken in Field during Dental Calculus Sampling

Sample	Cemetery	Burial	Sex	Age	Tooth	Surface	# of Controls Taken	Control Label*	Date Sampled	Comment
1							1	PT 1	July 22-July 29, 2013	Particle trap put out July 22 and collected July 29; before any samples were taken
2	Ust-Ida I	56-1	M	35-50	17	D. interprox	3	Box, Skull, PT 2	29/07/2013	
3	Ust-Ida I	38-1-1	M	35-45	32	M. interprox	4	Box/bag, Mandible, Weigh paper, PT 2	29/07/2013	
4	Ust-Ida I	33-1-1	U	12-15	25	Buccal	3	Paper bag, Maxilla, PT 2	29/07/2013	
5	Ust-Ida I	11-1-1	F	35-50	21	Lingual	4	Box, Maxilla, Weigh paper, PT 2	29/07/2013	NOT PROCESSED; NOT CALCULUS
6	Ust-Ida I	30-1-1	F	50+	45	Buccal	3	Bag/box, Mandible, PT 3	31/07/2013	Deposit is right above the CEJ. Listed as a score 1 but a borderline 2
7	Ust-Ida I	14-1-1	M	18-20	41	D. interprox	3	Bag/box, Mandible, PT 3	31/07/2013	Part of sample flew off during sampling so sample is quite small
8	Shamanka II	66-1	F	25-35	41	Buccal	4	Bag, Mandible, PT 4, Hands	05/08/2013	Edge of mesial interprox sampled as well; swabbed hands between sampling 8 and 9
9	Shamanka II	76-1	M	40-50	43	Buccal	4	Bag, Mandible, PT 4, Hands	05/08/2013	Deposit was located on the root; severe resorption
10	Lokomotiv	L-20-2-1	M	35-50	18	Buccal	4	Box, Maxilla, Ledge Surface, PT 5	10/08/2013	Thick deposit; piece contacted ledge (swabbed); lacquer on surfacepart of deposit, abraded with wire brush
11	Lokomotiv	L-16-1-1	M	45-55	45	Lingual	3	Bag, Mandible, PT 5	10/08/2013	Thick deposit located along the CEJ and onto the enamel
12	Lokomotiv	R-11-1	F	20-25	23	M. interprox	4	Bag, Maxilla, Hands/Ledge, PT 5	10/08/2013	Had lunch before sampling therefore there is a hand control
13	Lokomotiv	R-14-1	M	30-39	35	Lingual	3	Bag, Mandible, PT 6	17/08/2013	Crown crumbled while sampling, put it back together with putty for picture
14	Lokomotiv	L-35-1-1	U	20+	31	M. interprox	3	Bag, Mandible, PT 6	17/08/2013	Deposit went into tube without touching weigh paper
15	Lokomotiv	L-38-2-1	F	35-45	43	M. interprox	3	Bag, Mandible, PT 6	17/08/2013	Deposit went into tube without touching weigh paper
16	Shamanka II	109-1	F	40-50	26	Buccal	3	Box, Maxilla, PT 7	19/08/2013	Sample did not touch weigh paper
17	Shamanka II	47-1	F	20-25	36	Lingual	4	Box, Mandible, Hands, PT 7	19/08/2013	Sample did not touch weigh paper
18	Shamanka II	47-1	F	20-25	41	M. interprox (oriented lingual)	4	Box, Mandible, Hands, PT 7	19/08/2013	Mesial surface sampled but the tooth is twisted therefore the mesial surface is located lingual; sample did not touch weigh paper; weak enamel surface
19	Shamanka II	30-1	M	35-50	46	Buccal	3	Box, Mandible, PT8	20/08/2013	Massive, very thick deposit; deposit extends around to mesial and distal surfaces a bit as well
20	Shamanka II	26-3	U	6-8	85	Lingual	3	Bag, Mandible, PT 9	22/08/2013	Did not touch weigh paper but was hard to transfer from tooth to tube

Sample	Cemetery	Burial	Sex	Age	Tooth	Surface	# of Controls Taken	Control Label*	Date Sampled	Comment
21	Shamanka II	46	M	25-29	15	Buccal	3	Box, Maxilla, PT 10	30/08/2013	Did not touch weigh paper; tooth 15 exhibits a large round smooth defect on its mesial surface with exposed pulp- may be evidence of tool use
22	Shamanka II	46	M	25-29	46	Lingual	3	Box, Mandible, PT 10	30/08/2013	Did not touch weigh paper; large thick deposit
23	Shamanka II	60-2	F	40-44	44	Lingual	3	Bag, Mandible, PT 10	30/08/2013	Did not touch weigh paper
24	Shamanka II	24	M	25-35	18	Buccal/d. interprox	3	Box, Maxilla, PT 10	30/08/2013	One piece fell on counter but it was freshly bleached
25	Ust-Ida I	6-1	M	35-50	15	D. interprox	3	Bag, Maxilla, PT 11	02/09/2013	Sample is split between two tubes; 1 of 2 is sample that did not touch weigh paper or counter; 2 of 2 fell on the counter
26	Ust-Ida I	45-1-1	M	22-30	32	M. interprox	3	Bag, Mandible, PT 11	02/09/2013	Sample did not touch weigh paper, small deposit
27	Shamanka II	67	U	7-9	75	Lingual	3	Bag, Mandible, PT 12	09/09/2013	Did not touch weigh paper; deposit is quite small, paintbrushes for controls were sterilized at the lab
28	Ust-Ida I	44-2	U	5-6	85	Lingual	3	Bag, Mandible, PT 12	09/09/2013	Did not touch weigh paper; deposit is very small, paintbrushes for controls were sterilized in the lab
29	Lokomotiv	R-15	F	20-35	36	Lingual	3	Bag, Mandible, PT 12	09/09/2013	Did not touch weigh paper
30	Lokomotiv	L-28-1-1	F	35-40	41	M. interprox	3	Bag, Mandible, PT 12	09/09/2013	Did not touch weigh paper, skull and mandible quite laquered but this deposit looked relatively untouched, paintbrushes for controls sanitized in the field
31	Lokomotiv	L-28-1-1	F	35-40	46	Lingual	3	Bag, Mandible, PT 12	09/09/2013	Did not touch weigh paper; deposit was laquered, brushed deposit with a wire brush before sampling; paintbrushes sanitized in the field
32							1	random weigh paper sample	09/09/2013	
33	Ust-Ida I	19-1	M	30-35	45	Lingual	3	Bag, Mandible, PT 12	09/09/2013	Did not touch weigh paper; deposit is quite small
34	Khuzhir-Nuge XIV	32	F	50+	27	Buccal	3	Bag, Maxilla, PT 13	13/09/2013	Enamel fragment with calculus sampled; sample was brushed very little with tooth brush but otherwise just went straight into the tube, paintbrushes sterilized in field
35	Khuzhir-Nuge XIV	35.2	U	8-10	26	Buccal	3	Bag, Maxilla, PT 13	13/09/2013	Some of deposit did touch weigh paper, did not come off nicely(quite powdery); paintbrushes sterilized in field
36	Khuzhir-Nuge XIV	33	U	3-5	55	Buccal	3	Bag, Mandible, PT 13	13/09/2013	No maxilla so sampled the mandible instead as a control; small deposit; paintbrushes sterilized in the field
37	Khuzhir-Nuge XIV	11	M	35-50	47	Lingual	3	Bag, Mandible, PT 13	13/09/2013	Large sample, came off really nicely without touching weigh paper, paintbrushes sterilized in the field
38	Khuzhir-Nuge XIV	11	M	35-50	41	m. interprox	3	Bag, Mandible, PT 13	13/09/2013	Part of enamel fragmented while sampling- the enamel pieces (2) are in the tube with some calc still on them; sterilization of paintbrushes done in the field
39	Khuzhir-Nuge XIV	52	U	25-35	45	Buccal	3	Bag, Mandible, PT 13	13/09/2013	A small piece of root fell off while sampling but the crown stayed intact; paintbrush sterilization took place in the field
40							1	random curity wipe sample	13/09/2013	
41	Khuzhir-Nuge XIV	27.1	M	35-50	26	Buccal	3	Bag, Maxilla, PT 14	14/09/2013	No issues, paintbrushes sterilized in the field
42	Khuzhir-Nuge XIV	35.1	PM	18-20	11	D. interprox	3	Bag, Maxilla, PT 14	14/09/2013	Did not touch weigh paper; was abraded with a paintbrush sterilized in the field b/c running short of tooth brushes

Sample	Cemetery	Burial	Sex	Age	Tooth	Surface	# of Controls Taken	Control Label*	Date Sampled	Comment
43	Khuzhir-Nuge XIV	34	M	25-35	35	Lingual/d. interprox	3	Bag, Mandible, PT 14	14/09/2013	Did not touch weigh paper; was abraded with a paintbrush sterilized in the field b/c running short of tooth brushes
44	Khuzhir-Nuge XIV	37.2	U	14-17	41	M. interprox	3	Bag, Mandible, PT 14	14/09/2013	Did not touch weigh paper; paintbrushes sterilized in the field; toothbrush used to abrade surface
45	Khuzhir-Nuge XIV	12	U	25-35	15	Buccal	3	Bag, Maxilla, PT 14	14/09/2013	Did not touch weigh paper; fragile deposit; a piece of the calc deposit contacted researcher (labelled 2 of 2); part sampled as usual (1 of 2); deposit was abraded with a paintbrush sterilized in the field
46	Lokomotiv	L-15-1-1	M	20-35	31	Lingual/m. interprox	3	Bag, Mandible, PT 14	14/09/2013	Deposit has ocre covering surface; did not touch weigh paper; paintbrushes sterilized in the field
47	Lokomotiv	L-9-1-1	F	20+	15	Buccal	3	Bag, Maxilla, PT 14	14/09/2013	Deposit was abraded with a wire brush b/c no more toothbrushes; paintbrushes sterilized in the field
48	Ust-Ida I	5-1-1	U	7-9	42	M. interprox	3	Bag, Mandible, PT 14	14/09/2013	This is to replace 11-1-1 which I no longer think is a calc deposit; abraded with a paintbrush (sterilized in the field) b/c no more toothbrushes; small deposit
49							1	random weigh paper sample	14/09/2013	Half of weigh paper
50							1	counter swab- wet	14/09/2013	Swab of counter after it has been bleached
51							1	counter swab- dry	14/09/2013	Swab of counter after it has been bleached and left to dry (about 1 hour)
52							1	PT 15	Sept 9- Sept 16 2013	Particle trap left out for one week on shelf

\*PT= Particle Trap



## **Appendix D: Controls Reference Collection**

Table D-1 Plant particle counts from Irkutsk State University Lab Controls

Cemetery	Burial	Control	Starch	Phytolith	Other
Lokomotiv	R-14-1	Mandible	0	0	
Lokomotiv	L-38-2-1	Bag	4	0	
Lokomotiv	L-9-1-1	Bag	1	0	Starch like particle
Lokomotiv	R-11-1	Maxilla	1	0	
Lokomotiv	R-11-1	Particle trap	0	0	Blue fiber
Lokomotiv	L-15-1-1	Bag	50+	0	Unknown starch like particle
Lokomotiv	L-35-1-1	Mandible	1	0	
Lokomotiv	L-28-1-1	Particle trap	0	0	Red brown fiber
Lokomotiv	L-28-1-1	Mandible	9	0	Pollen, polyhedral particle
Lokomotiv	L-28-1-1	Bag	65+	0	Pink fibers
Lokomotiv	L-20-2-1	Maxilla	1	0	
Lokomotiv	L-20-2-1	Particle trap	0	0	Blue fiber
Lokomotiv	L-20-2-1	ledge	1	0	
Lokomotiv	L-16-1-1	Particle trap	0	0	Blue fiber
Lokomotiv	R-15-1	Particle trap	0	0	Red brown fiber
Lokomotiv	R-15-1	Bag	2?	0	Red brown fiber
Khuzhir-Nuge XIV	52	Mandible	0	1	
Khuzhir-Nuge XIV	27-1	Bag	0	0	
Khuzhir-Nuge XIV	27-1	Particle trap	0	0	Blue fiber, brown fiber
Khuzhir-Nuge XIV	35-2	Bag	1?	0	
Khuzhir-Nuge XIV	37-2	Mandible	3	3-4	Possible plant particle
Khuzhir-Nuge XIV	37-2	Particle trap	0	0	Blue fiber, brown fiber
Khuzhir-Nuge XIV	33	Mandible	5	1	Cells, pollen
Khuzhir-Nuge XIV	12	Maxilla	2+1?	1	Cells, unknown opaque double ringed particle
Khuzhir-Nuge XIV	12	Particle trap	0	0	Blue fiber, brown fiber
Khuzhir-Nuge XIV	35-1	Particle trap	0	0	Blue fiber, brown fiber
Khuzhir-Nuge XIV	35-1	Maxilla	0	1?	Pollen
Khuzhir-Nuge XIV	35-1	Bag	2+1?	0	
Khuzhir-Nuge XIV	34	Particle trap	0	0	Blue fiber, brown fiber
Khuzhir-Nuge XIV	34	Mandible	2	1	Blue fiber

Cemetery	Burial	Control	Starch	Phytolith	Other
Khuzhir-Nuge XIV	32	Bag	1	0	
Khuzhir-Nuge XIV	11	Bag	2	0	Red fiber
Shamanka II	47-1	Mandible	13+	0	
Shamanka II	47-1	Bag	8	0	
Shamanka II	47-1	Particle trap	0	0	
Shamanka II	60-2	Mandible	0	0	
Shamanka II	60-2	Bag	13	0	
Shamanka II	60-2	Particle Trap	0	0	
Shamanka II	23-1	Particle Trap	0	0	
Shamanka II	23-1	Mandible	1	0	
Shamanka II	46-1	Box	9	0	
Shamanka II	46-1	Mandible	18	0	
Shamanka II	46-1	Particle trap	0	0	
Shamanka II	24	box	4	0	
Shamanka II	24	Mandible	19+1?	0	
Shamanka II	24	Particle trap	0	0	
Shamanka II	76-1	Mandible	17+1	0	
Shamanka II	66-1	Mandible	2	0	
Shamanka II	66-1	Box	3	0	Spore/mold
Shamanka II	26-3	Particle trap	0	0	
Shamanka II	26-4	Mandible	3+2?	0	
Shamanka II	109-1	Particle trap	0	0	
Shamanka II	67	Bag	20	1	Fungal spore
Shamanka II	67	Particle trap	0	0	Red brown fiber
Ust'-Ida I	14-1-1	Bag/box	3	0	Helical particle, round starch like particle
Ust'-Ida I	6-1-1	Maxilla	3	0	Likely spore
Ust'-Ida I	6-1-1	Particle trap	0	0	Light blue fiber
Ust'-Ida I	38-1-1	Mandible	0	0	
Ust'-Ida I	38-1-1	Bag/box	5	0	Blue fibers
Ust'-Ida I	45-1-1	Particle trap	0	0	Light blue fiber
Ust'-Ida I	33-1-1	Maxilla	1+1?	1?	
Ust'-Ida I	44-2	Mandible	7	2	Sunburst round spore
Ust'-Ida I	44-2	Particle trap	0	0	Red brown fiber
Ust'-Ida I	19-1	Bag	14	1	Pollen
Ust'-Ida I	19-1	Particle	0	0	Red-brown fiber

Cemetery	Burial	Control	Starch	Phytolith	Other
		trap			
Ust'-Ida I	5-1-1	Particle	0	0	Blue fiber, brown fiber
		trap			
Ust'-Ida I	56-1	Box	2	0	
Ust'-Ida I	30-1-1	Mandible	4	0	Likely spore fragments

?=probable



Figure D-1. Phytoliths found within control swabs taken in the Irkutsk State University laboratory, scale=20  $\mu\text{m}$ : (a) Khuzhir-Nuge XIV Burial 52, mandible; (b) Khuzhir-Nuge Burial XIV 37-2, mandible; (c) Khuzhir-Nuge XIV Burial 37-2, mandible; (d) Ust'-Ida I Burial 44-2, bag; (e) Khuzhir-Nuge XIV Burial 37-2, mandible; (f) Ust'-Ida I Burial 19-1, bag; (g) Ust'-Ida I Burial 44-2, bag; (h) Shamanka II Burial 67, bag; (i) Khuzhir-Nuge XIV Burial 34, mandible.

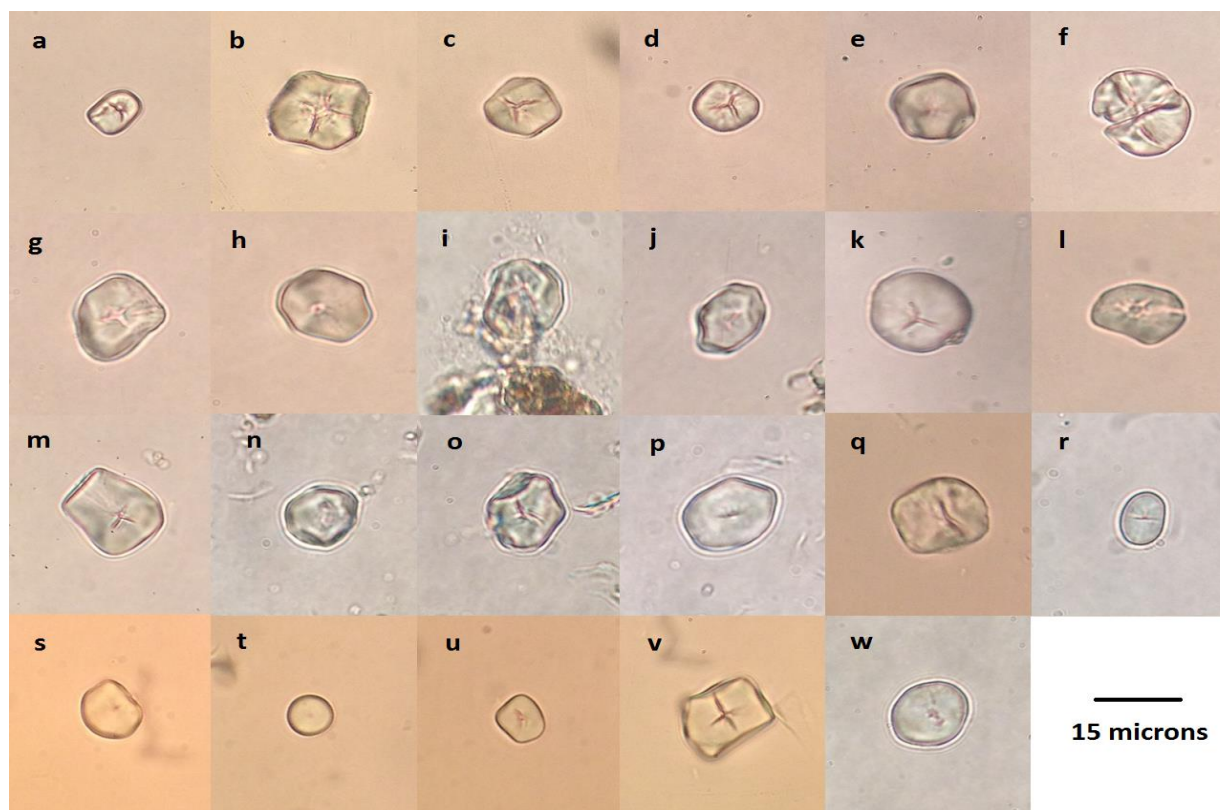


Figure D-2 Starch grains from control swabs taken at the Irkutsk State University laboratory (1 of 4): (a) Shamanka II Burial 46, box; (b) Shamanka II Burial 46, box; (c) Shamanka II Burial 46, box; (d) Shamanka II Burial 21-2, mandible; (e) Shamanka II Burial 21-2, mandible; (f) Shamanka II Burial 46, mandible; (g) Shamanka II Burial 5-1, bag; (h) Shamanka II Burial 5-1, bag; (i) Ust'-Ida I Burial 38-1-1, bag/box; (j) Shamanka II Burial 5-1, bag; (k) Shamanka II Burial 5-1, bag; (l) Shamanka II Burial 46, mandible; (m) Shamanka II Burial 5-1, bag; (n) Lokomotiv Burial L-28-1-1, bag; (o) Lokomotiv Burial L-28-1-, bag; (p) Lokomotiv Burial L-28-1-1, bag; (q) Lokomotiv Burial L-28-1-1, mandible; (r) Ust'-Ida I Burial 53-1-1, bag; (s) Lokomotiv Burial L-28-1-1, mandible; (t) Lokomotiv Burial 28-1-1, mandible; (u) Lokomotiv Burial L-28-1-1, mandible; (v) Lokomotiv Burial L-28-1-1, mandible; (w) Ust'-Ida I Burial 38-1-1, bag/box.

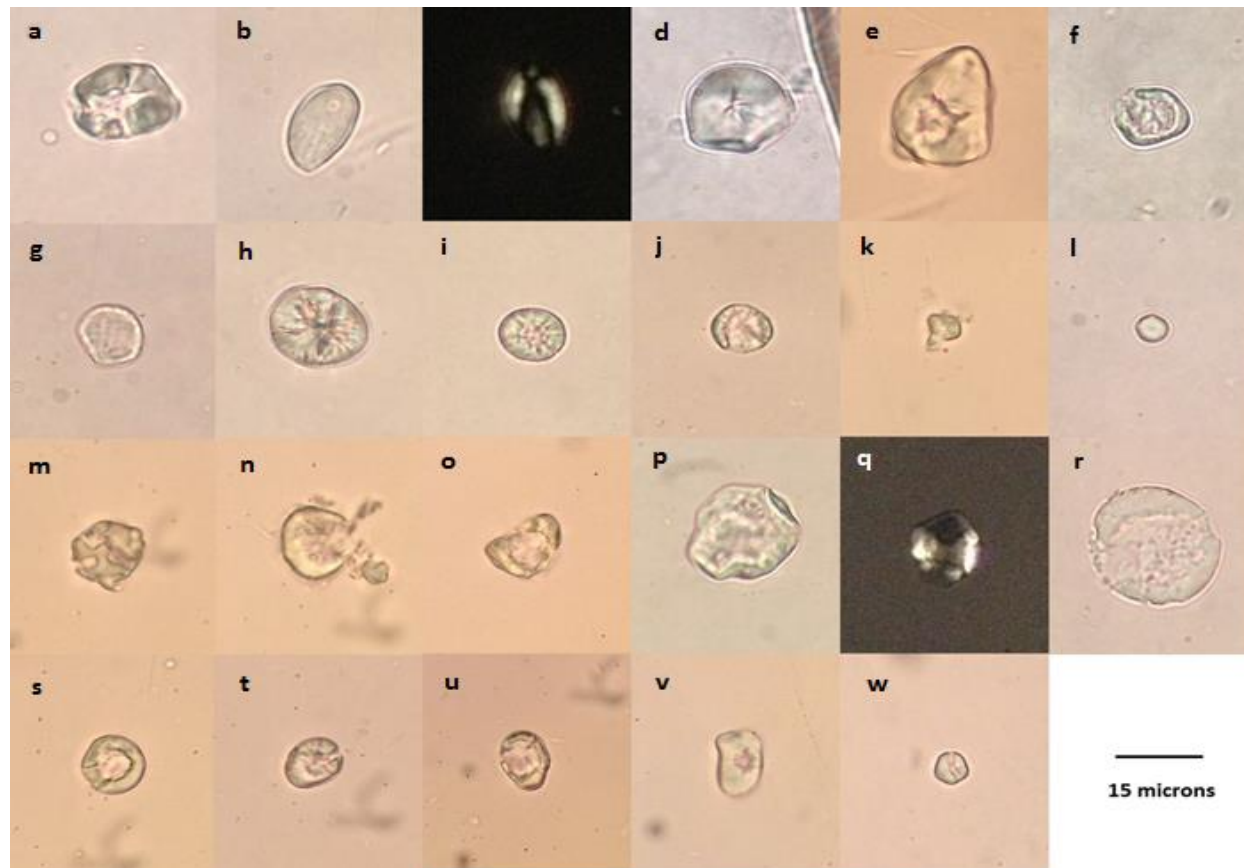


Figure D-3. Starch grains from control swabs taken at the Irkutsk State University laboratory (2 of 4): (a) Shamanka II Burial 5-1, bag; (b,c) Shamanka II Burial 5-1, bag; (d) Ust'-Ida I Burial 30-1-1, mandible; (e) Lokomotiv Burial L-15-1-1, bag; (f) Lokomotiv Burial L-28-1-1, bag; (g) Shamanka II Burial 46, mandible; (h) Shamanka II Burial 46, box; (i) Shamanka II Burial 46, box; (j) Shamanka II Burial 46, box; (k) Shamanka II Burial 30-1, mandible; (l) Shamanka II Burial 26-3, mandible; (m) Shamanka II Burial 47-1, bag; (n) Shamanka II Burial 47-1, bag; (o) Shamanka II Burial 47-1, bag; (p,q) Shamanka II Burial 46, mandible; (r) Shamanka II Burial 5-1, bag; (s) Shamanka II Burial 47-1, mandible; (t) Shamanka II Burial 47-1, mandible; (u) Shamanka II Burial 47-1, mandible; (v) Shamanka II Burial 60-2, bag; (w) Shamanka II Burial 24, box.

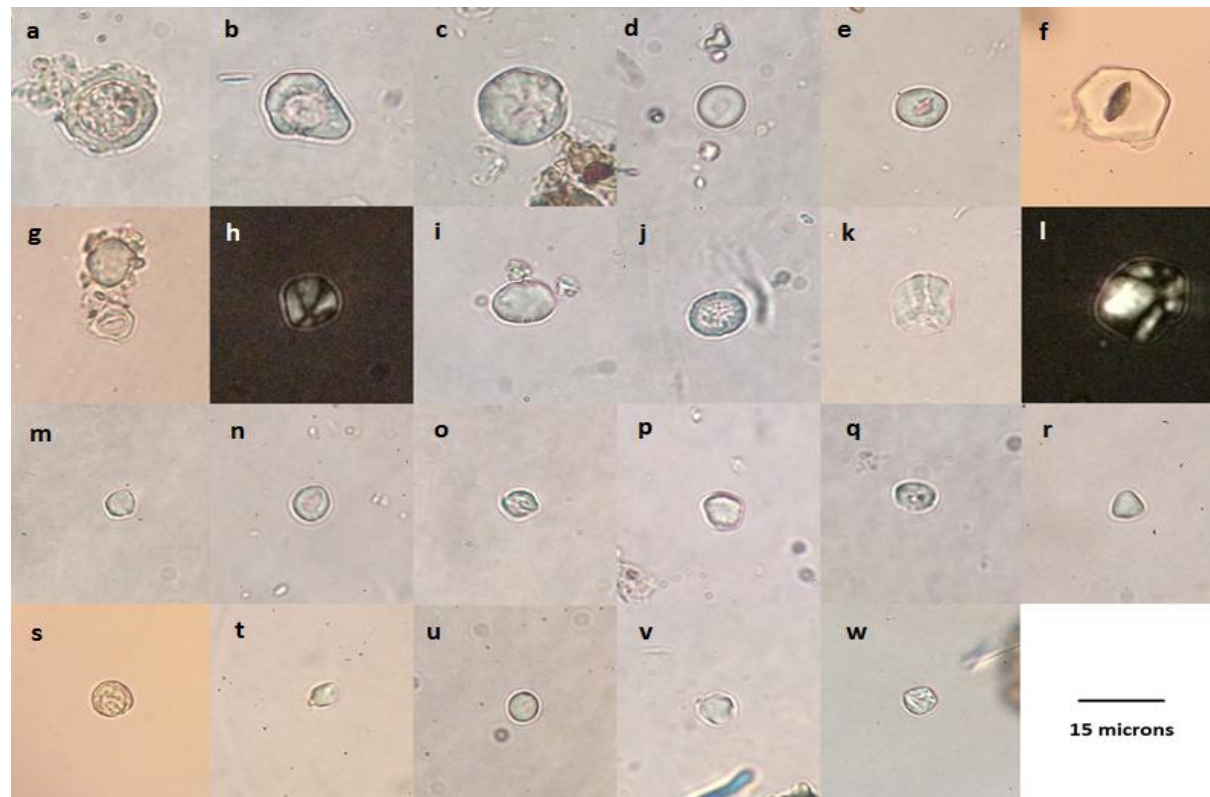


Figure D-4. Starch grains from control swabs taken at the Irkutsk State University laboratory (3 of 4): (a) Ust'-Ida I Burial 30-1-1, mandible; (b) Lokomotiv Burial L-28-1-1, bag; (c) Khuzhir-Nuge XIV Burial 37-2, mandible; (d) Ust'-Ida I Burial 53-1-1, bag; (e) Ust'-Ida I Burial 44-2, bag; (f) Lokomotiv Burial L-38-2-1, bag; (g) Ust'-Ida I Burial 6-1-1, maxilla; (h) Shamanka II Burial 26-3, mandible; (i) Ust'-Ida I Burial 38-1-1, bag/box; (j) Ust'-Ida I Burial 53-1-1, bag; (k) Khuzhir-Nuge XIV Burial 35-1, bag; (l) Lokomotiv Burial L-28-1-1, bag; (m) Lokomotiv Burial L-20-2-1, ledge; (n) Ust'-Ida I Burial 44-2, bag; (o) Lokomotiv Burial L-20-2-1, ledge; (p) Ust'-Ida I Burial 44-2, bag; (q) Ust'-Ida I Burial 53-1-1, bag; (r) Ust'-Ida I Burial 30-1-1, mandible; (s) Lokomotiv Burial L-38-2-1, bag; (t) Ust'-Ida I Burial 14-1-1, mandible; (u) Ust'-Ida I Burial 30-1-1, mandible; (v) Khuzhir-Nuge XIV Burial 37-2, mandible; (w) Khuzhir-Nuge XIV Burial 35-1, bag.



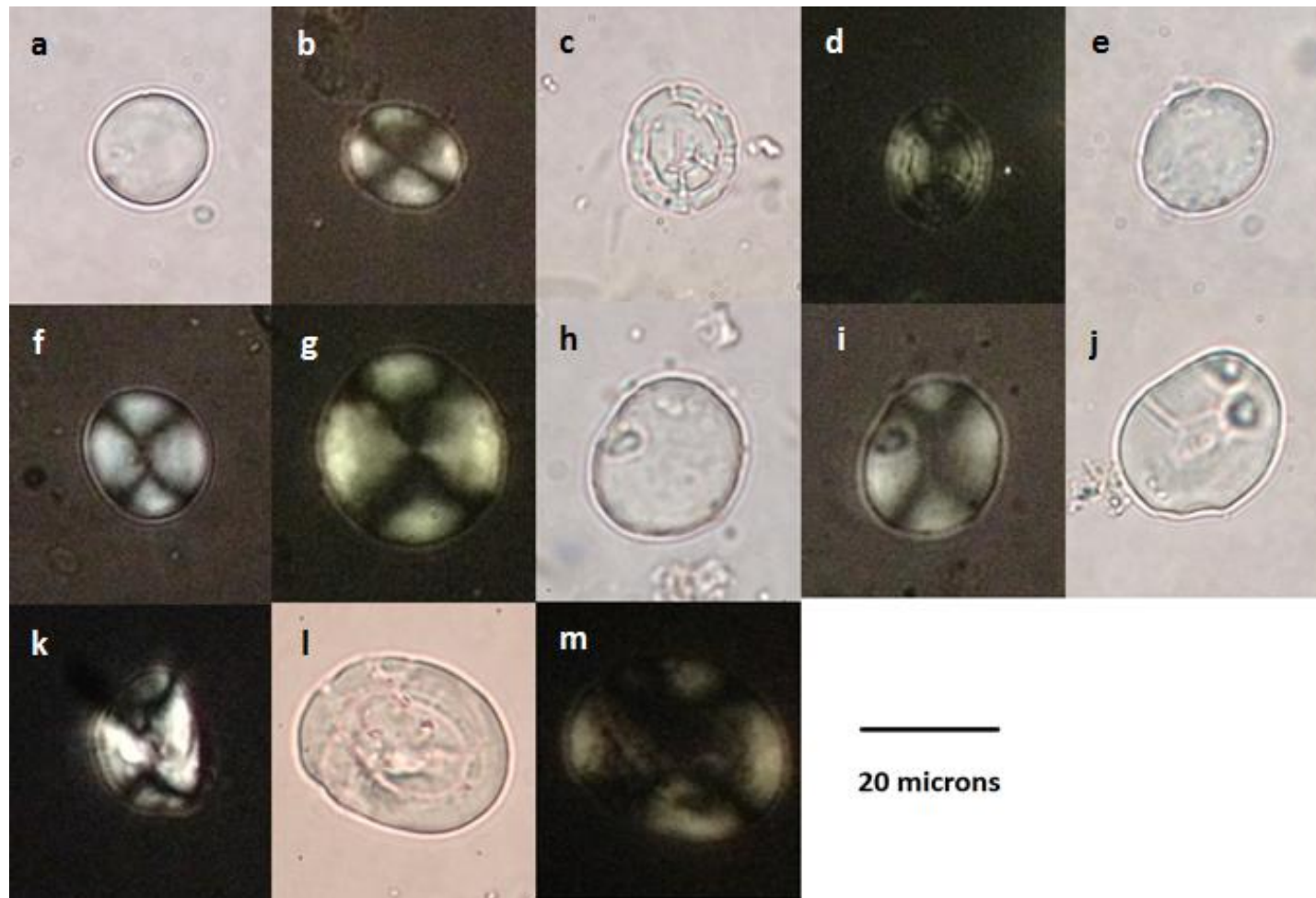


Figure D-5. Starch grains from control swabs taken at the Irkutsk State University laboratory (4 of 4): (a) Ust'-Ida I Burial 33-1-1, maxilla; (b) Ust'-Ida I Burial 44-2, bag; (c,d) Ust'-Ida I Burial 53-1-1, bag; (e) Ust'-Ida I Burial 14-1-1, bag/box; (f) Ust'-Ida I Burial 53-1-1, bag; (g) Khuzhir-Nuge XIV Burial 33, mandible; (h,i) Khuzhir-Nuge XIV Burial 33, mandible; (j) Lokomotiv Burial L-20-2-1, maxilla; (k) Lokomotiv Burial L-20-2-1, maxilla; (l,m) Shamanka II Burial 5-1, bag. Note that although many of these have a similar shape, their extinction crosses are often not the same and therefore these starch grains likely represent a variety of plant genera.

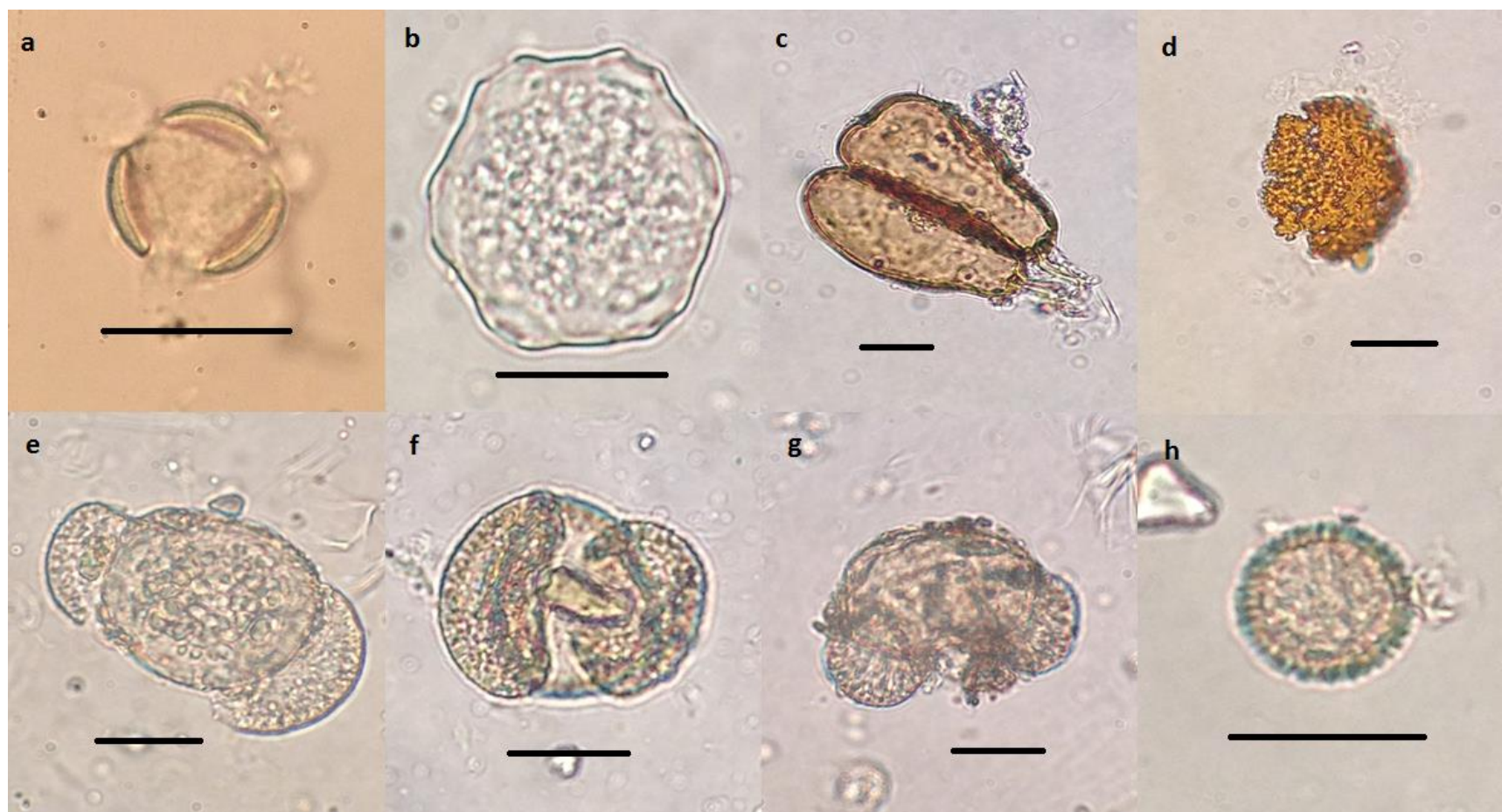


Table D-6. Pollen and spores found in control swabs from the Irkutsk State University laboratory, scale=20  $\mu\text{m}$ : (a) Lokomotiv Burial L-28-1-1, mandible; (b) Khuzhir-Nuge XIV Burial 33, mandible; (c) Khuzhir-Nuge XIV Burial 35-1, maxilla; (d) Ust-Ida I Burial 30-1-1 mandible; (e) Khuzhir-Nuge XIV Burial 33, mandible; (f) Khuzhir-Nuge XIV Burial 35-1, maxilla; (g) Ust'-Ida I Burial 53-1-1, bag; (h) Ust'-Ida I Burial 44-2, bag.

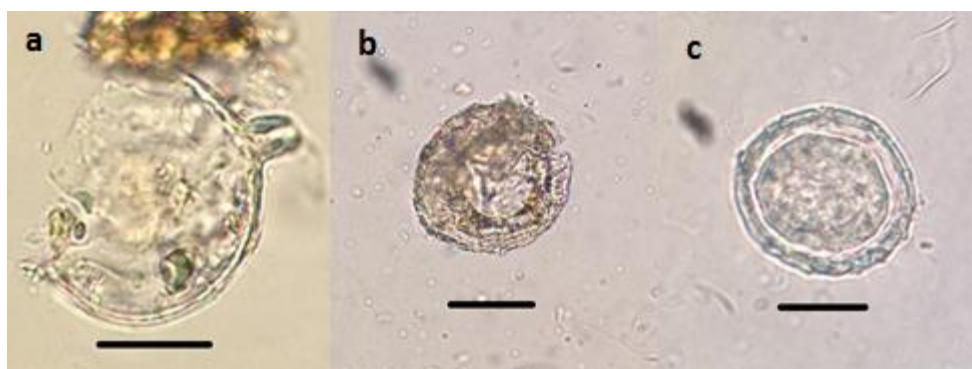


Figure D-7. Other and unknown particles found in control swabs from Irkutsk State University laboratory, scale=20  $\mu\text{m}$ : (a) Unknown particle from Khuzhir-Nuge XIV Burial 37-2 (mandible) that may be a fungal in origin; (b) Possible testate amoeba test from Khuzhir-Nuge XIV Burial 33, mandible; (c) Unknown opaque ringed particle from Khuzhir-Nuge XIV Burial 12, maxilla.



Figure D-8 Mold spores and fungal activity within control swabs from Irkutsk State University laboratory, scale=30  $\mu\text{m}$ : (a) Khuzhir-Nuge XIV Burial 11, bag; (b) Khuzhir-Nuge XIV Burial 27-1, bag; (c) Shamanka II Burial 66-1, bag; (d) Ust'-Ida I Burial 44-2, bag; (e) Shamanka II Burial 67, bag.



Table D-2 Plant Particle Counts within Particle Traps from University of Saskatchewan Lab

Particle Trap	Round	Burials	Starch	Phytoliths	Other
1	1	LOK: R-14-1, L-38-2-1	0	0	
2	2	K14: 35.2, 27.1, 52	2	0	
3	3	LOK: L-28-1-1, L-15-1-1, R-11-1	0	0	
4	4,5	SHA: 47-1 (2 of 2), 30-1, 60-2, 46 (1 of 2), 76-1, 24	0	0	
5	6,7	SHA: 30-1, 66-1, 47-1 (1 of 2), 109-1, 67, 26-3	0	0	Pink fiber
6	8,9,10	UID: 14-1-1, 6-1-1, 38-1-1, 45-1-1, 19-1, 33-1-1, 44-2; K14: 37.2, 12, 33	0	0	Blue and clear fibers
7	11,12,13	LOK: L-35-1-1, L-20-2-1, L-9-1-1 (1 of 2); UID: 56-1, 30-1-1, 5-1-1; K14: 34, 12, 35.1	5	0	Blue fiber
8	14,15,16	LOK: R-15-1, L-16-1-1, L-9-1-1; SHA: 46 (2 of 2); K14: 11 (1 of 2), 32	0	0	

LOK= Lokomotiv, SHA= Shamanka II, UID= Ust'-Ida I, K14= Khuzhir-Nuge XIV

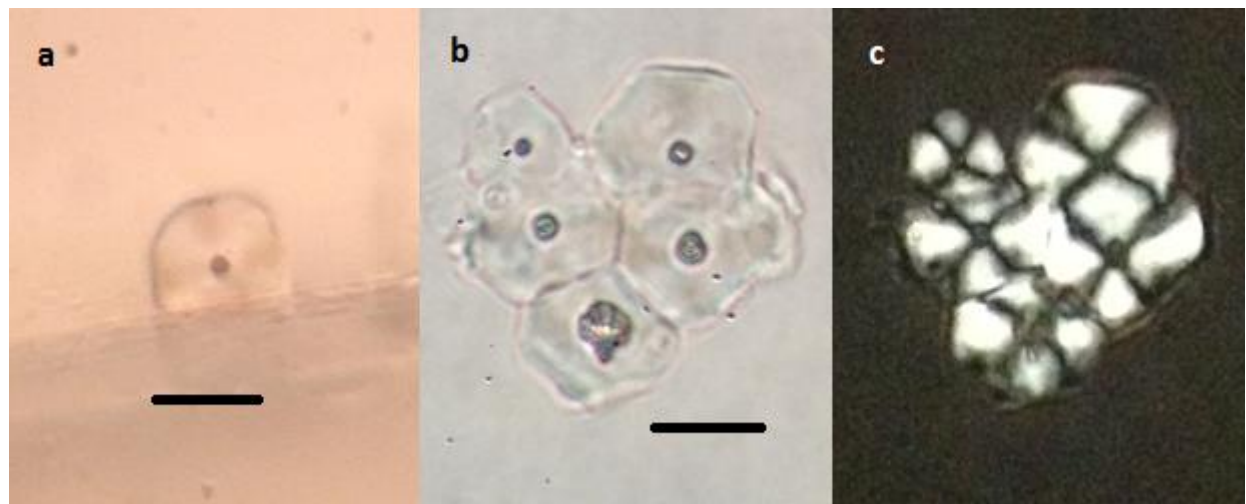


Figure D-9. Starch grains with open hila found in particle traps from the University of Saskatchewan laboratory, scale=10 µm: (a) Round 2 particle trap; (b,c) Rounds 11, 12, and 13 particle trap.

Table D-3 Plant Particle Counts for Blank Controls

Blank Control	Burials	Starch	Phytoliths	Other
1	LOK: R-14-1, L-38-2-1	7	0	
2	K14: 35-2, 27-1, 52	1	0	
3	LOK: L-28-1-1 (2 of 2), L-15-1-1, R-11-1	1	0	
4	SHA: 47-1 (2 of 2), 30-1, 60-2	0	0	
5	SHA: 46 (1 of 2), 76-1, 24	0	0	
6	SHA: 30-1, 66-1, 47-1 (1 of 2)	1	0	
7	SHA: 109-1, 67, 26-3	0	0	
8	UID: 14-1-1, 6-1-1, 38-1-1	0	0	
9	UID: 45-1-1, 19-1, 33-1-1, 44-2	1-2	0	
10	K14: 37-2, 12, 33	1	0	
11	LOK: L-35-1-1, L-20-2-1, L-28-1-1 (1 of 2)	1	0	
12	UID: 56-1, 30-1-1, 5-1-1	1	0	Black fiber
13	K14: 34, 12, 35-1	0	0	
14	LOK: R-15-1, L-16-1-1, L-9-1-1	5	0	
15	SHA: 46 (2 of 2)	0	0	
16	K14: 11 (1 of 2), 32	1	0	

LOK= Lokomotiv, SHA= Shamanka II, UID= Ust'-Ida I, K14= Khuzhir-Nuge XIV

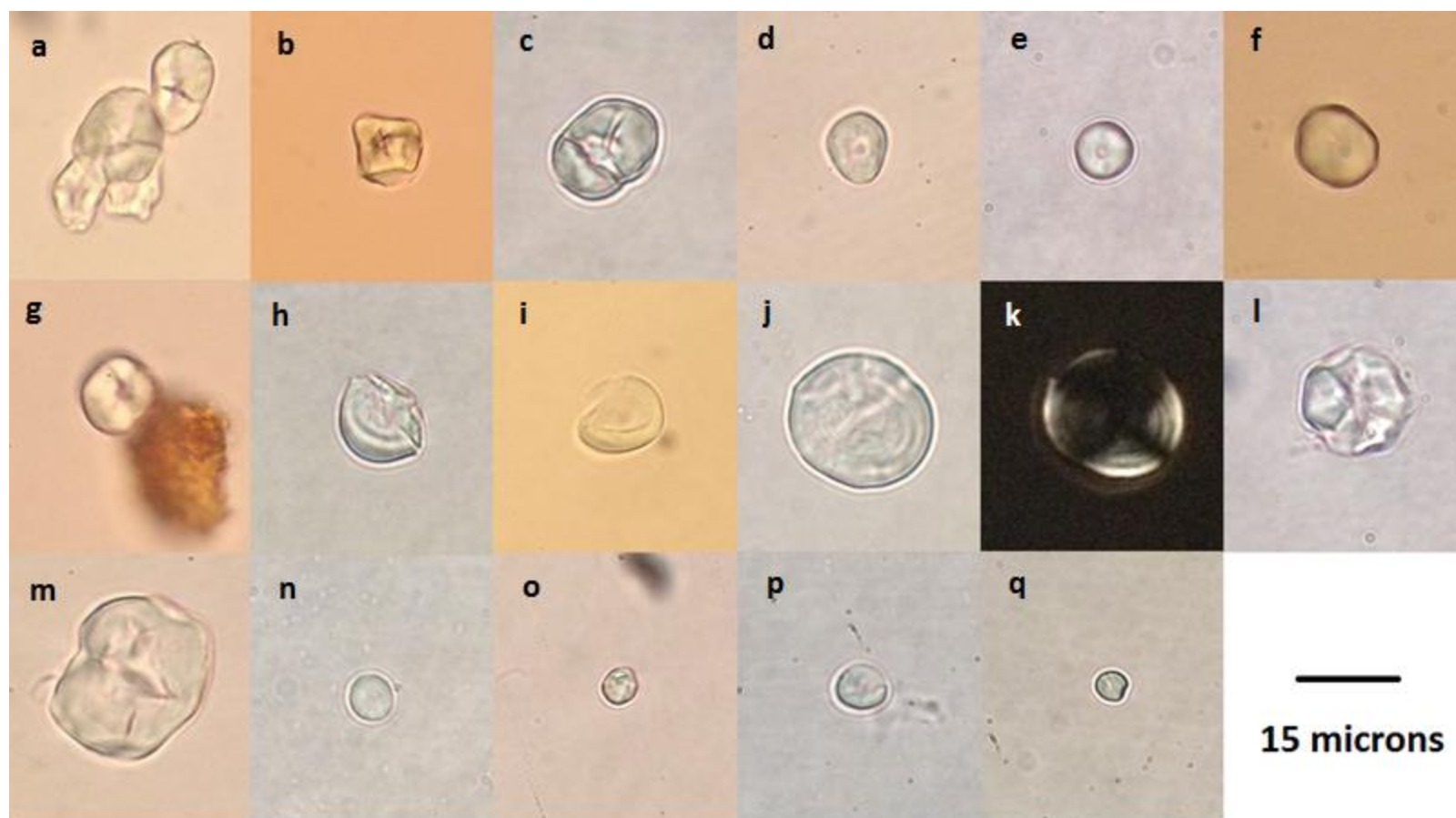


Figure D-10. Starch grains found in blank controls from the University of Saskatchewan laboratory: (a,b) Round 1; (c) Round 12; (d) Round 9; (e) Round 14; (f) Round 3; (g) Round 1; (h) Round 16; (i) Round 10; (j,k) Round 11; (l) Round 14; (m,n,p,q) Round 9; (o) Round 6.

## **Appendix E: Microscopic Analysis of Dental Calculus Summaries by Individual**

Note: Please see Appendix F for definitions on various shapes discussed below. “Subsamples” refer to different aliquots drawn from the same processed sample while “samples” refer to different processed calculus deposits taken from the same individual or two parts of the same deposit processed on different occasions.

### *Lokomotiv Burials*

#### R-14-1

Two subsamples were examined from the lingual side of the left mandibular second premolar of Lokomotiv Burial R-14-1, an adult male, aged 30-39. As mentioned in Chapter 3, some of the original sample was lost when the *Lycopodium* tablet was added. Due to loss of sample, a lycopod spike was not used to determine sample count. No plant particles were found within subsample one (see Chapter 3, Section 3.2.3 and 3.2.4 for subsampling methodology). A piece of what looked like plant tissue was found in subsample two but may have had its origins from the soil rather than the calculus. The sample was very small and may account for the lack of observed particles. The sample was associated with the contaminated blank control from the first round of sample processing and preparation in the University of Saskatchewan lab. Since no plant particles were found it was not a concern.

#### L-38-2-1

Two subsamples were examined from the mesial interproximal surface of the right mandibular canine of Lokomotiv Burial L-38-2-1. Lokomotiv Burial L-38-2-1 was an adult female, aged 35 to 45. No *Lycopodium* tablet was added after the R-14-1 sample overflowed. Nothing was found within the first subsample. The second subsample contained one small starch grain (Figure E-1). The starch grain was found along the edge of the coverslip, underneath the layer of nail polish used to seal the slide. The starch grain could be rotated though so it was unlikely that the starch grain originated from the nail polish. The starch grain was 11.6 µm in length and was round in shape. Under cross polarized light it was seen to have a central hilum; a small surface indentation was located over it. The starch grain was similar to some *Zea mays* grains observed in comparative slides and therefore likely was a contaminant. This sample was also associated with the contaminated blank control from the first round of attempted sample



processing and preparation in the University of Saskatchewan lab, which may explain where the contaminant came from.

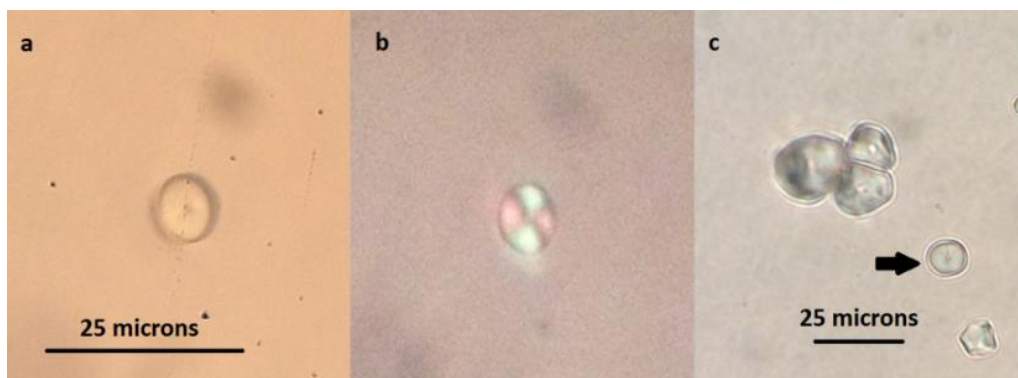


Figure E-1. Starch grain recovered from calculus associated with Lokomotiv Burial L-38-2-1: (a) Starch grain found in calculus subsample; (b) same starch grain under cross polarized light, scale the same as (a); (c) starch grain from *Zea mays* with similar characteristics.

#### L-28-1-1

Lokomotiv Burial L-28-1-1 was an adult female, aged 35- 40. Two samples were taken from Lokomotiv Burial L-28-1-1: one from the mesial surface of the right central mandibular incisor (sample one) and one from the lingual side of the right mandibular first molar (sample two). The subsample extracted from sample one contained two very similar starch grains. Both were very small and appeared to be roundish in shape, though they could not be fully rotated under the coverslip to be certain of the three-dimensional shape. One measured 4.7  $\mu\text{m}$  in length and seemed to have a central hilum (Figure E-2a, b) while the other, which was attached to demineralized calculus, measured 3.6  $\mu\text{m}$  in length (Figure E-2c, d). Both were very small and it was hard to make out any defining features, except a possible indentation at the hilum. A few starch grains with similar size and indentations at the hilum were noted in controls (Appendix D), but the starch grain embedded within the demineralized calculus was not dislodged by tapping the coverslip, suggesting an ancient origin. Since the two starch grains were almost identical, both were retained within the analysis. Fungal activity was noted, in the form of brown and opaque hyphae and small brown spores, as well as one unknown reddish brown possible spore found embedded within the calculus.

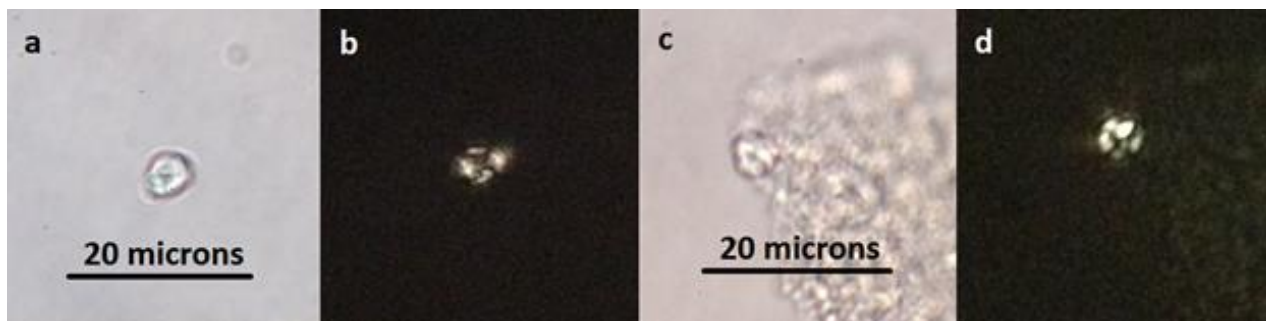


Figure E-2. Starch grains recovered from calculus associated with Lokomotiv Burial L-28-1-1, sample one, scale applies to all pictures: (a,c) Starch grains under normal light; (b,d) Starch grains under cross polarized light.

The subsample from sample two contained one starch grain that appeared to be damaged (Figure E-3a, b). It looked dehydrated or collapsed, based on the shrunken look to the exterior of the grain and warped extinction cross. The extinction cross was still visible though and indicated a central hilum. It was round in shape and measured 9.5  $\mu\text{m}$  in length. A similar dehydrated-looking starch grain was found within a swab analyzed from the bag the mandibular teeth were kept in (Figure E-3c, d); therefore the starch grain was considered a contaminant. A few brown cells/spores were observed in a chain within a piece of demineralized calculus; they were likely fungal or microbial in origin.

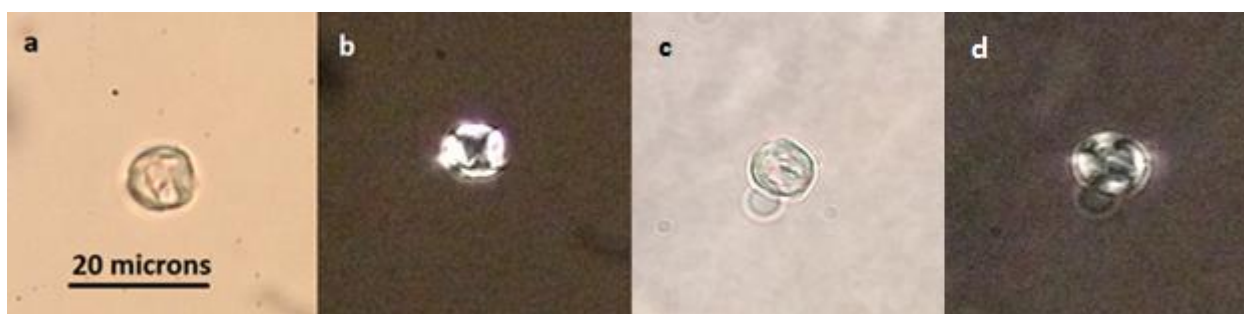


Figure E-3. Starch grain recovered from calculus associated with Lokomotiv Burial L-28-1-1, sample two, and a comparative contaminant, scale applies to all pictures: (a,b) Sample starch grain under normal light and cross polarized light respectively; (c,d) Contaminant starch grain from bag control under normal light and cross polarized light respectively.

### R-11-1

Lokomotiv Burial R-11-1 was an adult female, aged 20-25, and the mesial interproximal surface of the left maxillary canine was examined. No starch grains were found within the subsample. Three other particles were found and may represent possible phytoliths. One was a pinkish colour, with a hook shape similar to a trichome, but could not be rotated to see its three-dimensional structure. It was 13.3  $\mu\text{m}$  in length (Figure E-4a). The other two were opaque in appearance and could not be moved either. One was shaped like a “chef’s hat” with an inclusion/void. It measured 10  $\mu\text{m}$  (Figure E-4b). The other had an unknown shape, as it was embedded in the calculus and partially blocked from view, but it also appeared to have an inclusion/void present and the entire particle measured  $\sim 11$   $\mu\text{m}$  in length (Figure E-4c). The sample from Lokomotiv Burial R-11-1 was dominated by the presence of clumps of small pollen aggregates (Glenn Stuart, personal communication 2014; Figure E-5). Other clumps of brown particles appeared to have little structure other than the outer wall of the particle and may have represented fungal spores rather than pollen.

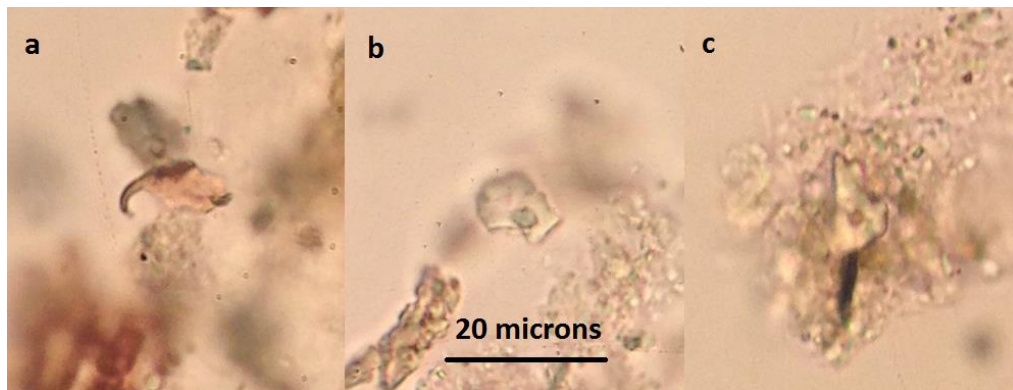


Figure E-4. Possible phytoliths recovered from calculus associated with Lokomotiv Burial R-11-1, scale applies to all pictures: (a) Trichome-like particle; (b,c) Opaque particles with inclusions present.

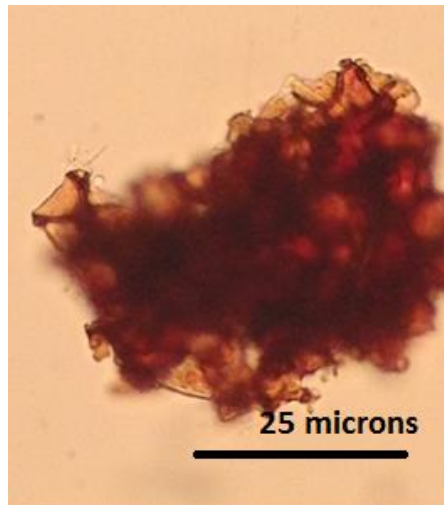


Figure E-5. Clump of pollen recovered from calculus associated with Lokomotiv Burial R-11-1.

#### L-15-1-1

Calculus from the mesial interproximal and lingual surfaces of the left mandibular central incisor was sampled from Lokomotiv Burial L-15-1-1, an adult male, aged 20-35. Two subsamples were analyzed. The first subsample contained only small amounts of undissolved calculus material and was therefore deemed to be unrepresentative of the sample. No plant particles were seen. In the second subsample, two possible starch grains were observed. One of the starch grains was attached to another particle that had no birefringence (Figure E-6a) while the other was attached to a small amount of demineralized dental calculus (Figure E-6b). They were both very small ( $\sim 3\text{-}4\mu\text{m}$ ) and very few to no features were observable. Both appeared to exhibit extinction crosses, though neither was overly pronounced (Figure E-6c). Starch grains associated with controls for this individual did not match these starch grains, though some from other controls were quite similar. Both were attached to other material and were therefore kept within the analysis but should be regarded cautiously.

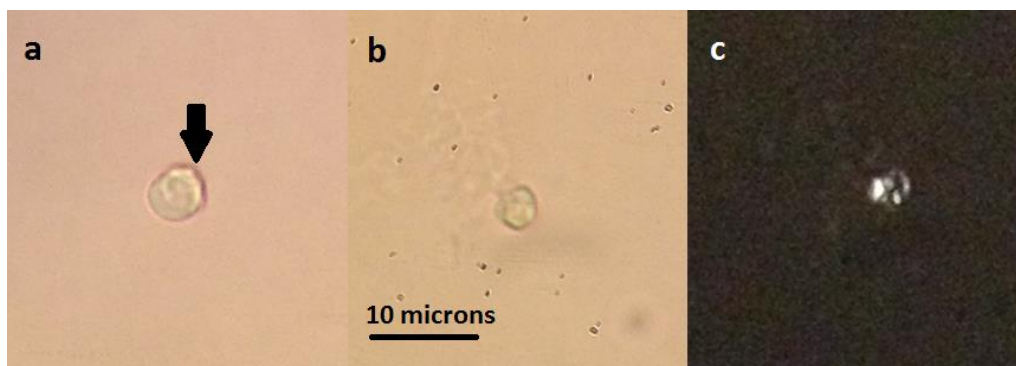


Figure E-6. Starch grains recovered from calculus associated with Lokomotiv Burial L-15-1-1, scale applies to all pictures: (a) Arrow points to the starch grain attached to another slightly larger particle; (b,c) Small starch grain attached to faint calculus material, under normal and cross polarized light respectively.

### L-35-1-1

Lokomotiv Burial L-35-1-1 was an adult of undetermined sex, over the age of 20. Calculus from the mesial interproximal surface of the left mandibular central incisor was analyzed. The subsample contained an abundance of brown circular cell-like particles, some of which were identified as possible pollen (Figure E-7). A few resemble some species of *Artemisia* (Glenn Stuart, personal communication 2014). Fungal activity was noted, in the form of brown hyphae and spores. A small, possibly circular, clear/opaque unknown particle was observed firmly attached to other material within the dental calculus. The particle could be moved but not rotated because of this attachment. It looked similar to a starch grain but had no birefringence. It exhibited a slight double border and was 6.5  $\mu\text{m}$  in length. One large starch grain was seen as well, measuring 23.1  $\mu\text{m}$  in length (Figure E-8a-c). It was concave-lenticular in shape when rotated (Figure E-8c). Five to seven concentric lamellae were visible on the surface of the starch grain and a central hilum was seen under cross polarized light. Arms of the extinction cross were wide and barely connected at the center (Figure E-8b). Lenticular starch grains were observed in controls, but none matched both the concavity and extinction cross seen in this example, therefore it was kept in the analysis.

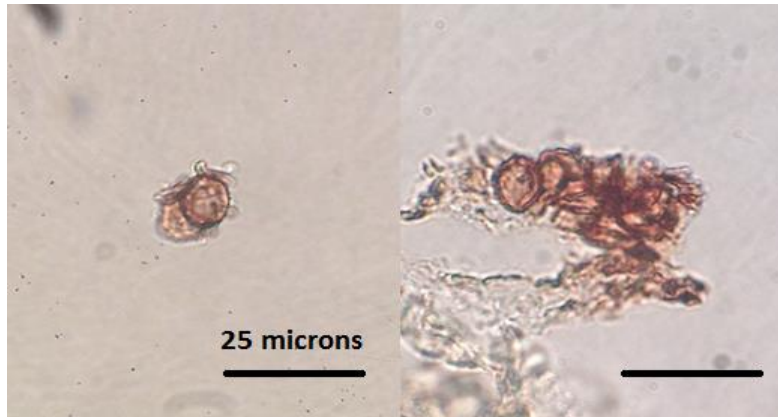


Figure E-7. Pollen recovered from calculus associated with Lokomotiv Burial L-35-1-1, scale in both pictures is 25  $\mu$ m. Tentatively identified at *Artemisia*.

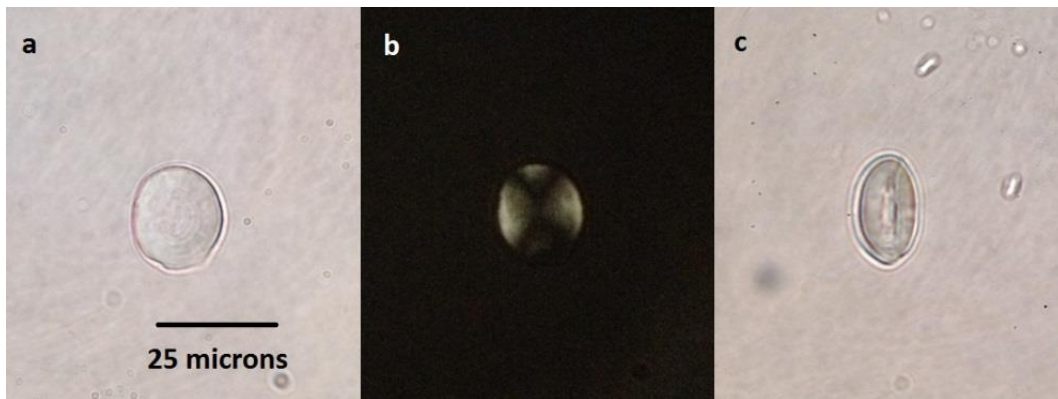


Figure E-8. Starch grain recovered from calculus associated with Lokomotiv Burial L-35-1-1, scale applies to all pictures: (a,b) View of starch grain under normal and cross polarized light respectively; (c) View of starch grain from the side, showing three-dimensional shape, under normal light.

#### L-20-2-1

The buccal surface of the right maxillary third molar was sampled from Lokomotiv Burial L-20-2-1, an adult male, aged 35-50. It was noted in the field that the sample from Burial L-20-2-1 may have had some lacquer on its surface, and the researcher attempted to abrade the surface to remove the lacquer. Despite this, it is the judgement of the researcher that some of the masses found within the sample upon analysis were likely chunks of lacquer, based on the presence of solid, transparent to opaque film fragments within the samples (see Section 5.2.1, Figure 5.3a). A number of particles were found within the subsample. First, a large unknown particle with what looked like a stem was observed (Figure E-9a). It was a light brown-opaque



color and appeared to be hollow in the middle. The stem, attached at one end, was similar to those seen on a number of spores observed with attached hyphae, though this particle was quite distinct from other spores noted.

There were two particles of unknown origin. One was similar in surface appearance to other minerals observed in these samples, but it had two large protrusions, making it quite distinct from other particles in this sample (Figure E-9b). It may also have been a product of the lacquer. It was 43.5  $\mu\text{m}$  in length. Another particle, measuring 34  $\mu\text{m}$ , consisted of a clear larger central round mass with two small round protrusions on either side of the central mass (Figure E-9c). Only the two protrusions displayed any kind of birefringence, though it was slight and did not form the cross typical of starches.

Two definite starch grains were identified, as well as four possible tiny starch grains (Figure E-10a-f and Figure E-11). One starch grain appeared oval to slightly concave-lenticular in shape, with a central hilum, though it could not be fully rotated to get a good look at its three-dimensional shape (Figure E-10c, d). There was a possible slight depression in the middle and the whole grain measured 18.7  $\mu\text{m}$  in length. The second starch grain was quite large, 24.3 $\mu\text{m}$ , and convex-lenticular in shape with six to seven concentric lamellae visible (Figure E-10e). Under cross polarized light, a central hilum was evident and the extinction cross arms were wide and barely connected in the middle (Figure E-10f). A similar starch grain was seen in a bag swab from Khuzhir-Nuge XIV Burial 33, but the extinction cross patterns were different. Three possible starch grains were noted, though their extremely small size made it hard to focus on and observe any characteristics. Two of these starch grains could not be seen under normal light. Precise measurements could not be taken but they were each likely less than 5  $\mu\text{m}$  in length. They appeared to be embedded within demineralized calculus and only faint possible extinction crosses could be made out under cross polarized light (Figure E-11). More grains may have been embedded deeper within the same calculus piece. All starch grains are considered to have originated from the calculus matrix rather than contamination due to direct association with calculus material and lack of similarities with reference contaminants. Despite this, blue and clear fibers were found within the sample, suggesting some contamination, possibly from the lacquer. Therefore, all particles within this sample should be regarded cautiously due to this possible contamination.



Figure E-9. Unknown particles recovered from calculus associated with Lokomotiv Burial 20-2-1, scale in all pictures is 25  $\mu$ m: (a) Unknown hollow particle with stem; (b) Opaque mineral-like particle with odd protrusions; (c) Round opaque to translucent particle with round side protrusions.

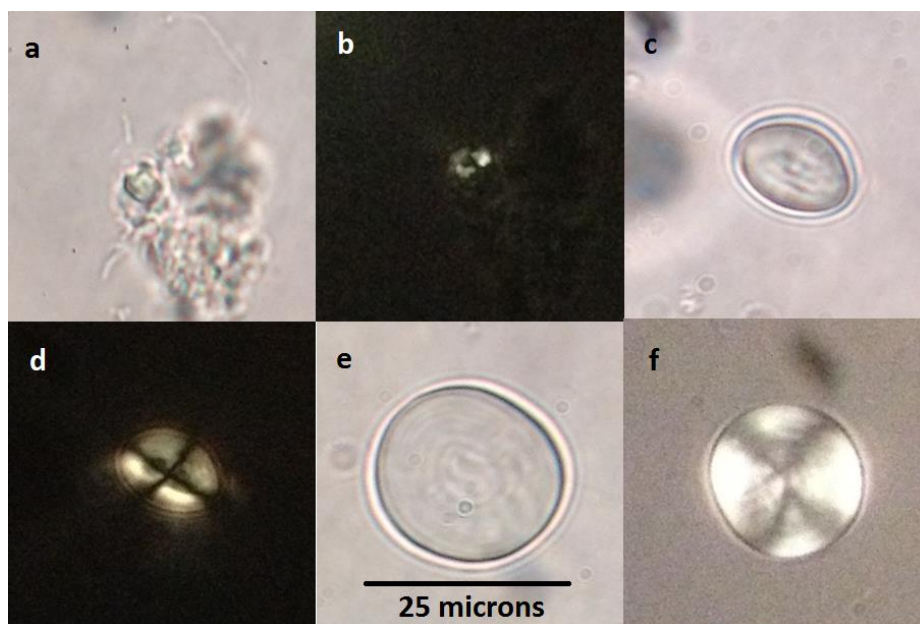


Figure E-10. Starch grains recovered from calculus associated with Lokomotiv Burial L-20-2-1, scale applies to all pictures: (a,b) Tiny possible starch grain, under normal and cross polarized light respectively, adhering to demineralized calculus; (c,d) Oval to slightly concave-lenticular shaped starch grain under normal and cross polarized light respectively; (e,f) Large lenticular starch grain with visible lamellae under normal and cross polarized light respectively.





Figure E-11. Possible starch grains (indicated by red arrows) recovered from calculus associated with Lokomotiv Burial L-20-2-1, under cross polarized light (600X magnification; grains are approximately 5  $\mu\text{m}$  or less in diameter).

#### L-16-1-1

Lokomotiv Burial L-16-1-1 was an adult male, aged 45-55. The subsample contained no phytoliths or starch grains. The sample was dominated by a very large number of brown cell-like particles. Their size and features were similar to Burials R-11-1 and L-35-1-1 but they were closely associated with brown hyphae; therefore, the particles were considered part of the fungal activity evident in the sample. Opaque, elongated hyphae occurred in abundance as well. Two organic fibers were found: one blue, most likely from modern clothing, and one striated and opaque, of unknown origin.

#### R-15-1

A sample was taken from the lingual surface of the left mandibular first molar, belonging to Lokomotiv Burial R-15-1, an adult female, aged 20-35. One starch grain and one possible phytolith were observed. The possible phytolith, 8.5  $\mu\text{m}$  in length, was attached to surrounding material and could not be moved to get an idea of its three-dimensional shape, although it appeared to be possibly trapezoidal (Figure E-12a). No inclusions or central voids were seen. The starch grain exhibited a rounded pyramidal/semi-circular shape, with a faceted bottom. It was 12  $\mu\text{m}$  in length, and had a small indentation at the hilum, which was centrally placed (Figure E-12b, c). The arms of the extinction cross were sharp. A starch grain with a double-

faceted bottom was found in a control from an Ust'-Ida I individual, but the extinction crosses of these two starch grain were distinct when compared. This grain was also associated with a contaminated blank control (round 14) and, while it was distinct from contaminants, it should be considered cautiously, even though it was kept in the analysis. An unknown rod-shaped particle with scalloped edges was also observed (Figure E-13). Under cross polarized light, no birefringence was seen but light refracted around the edges. It may have been a diatom. Lastly, an indigo particulate, likely associated with denim apparel, was present and was not ancient in origin.

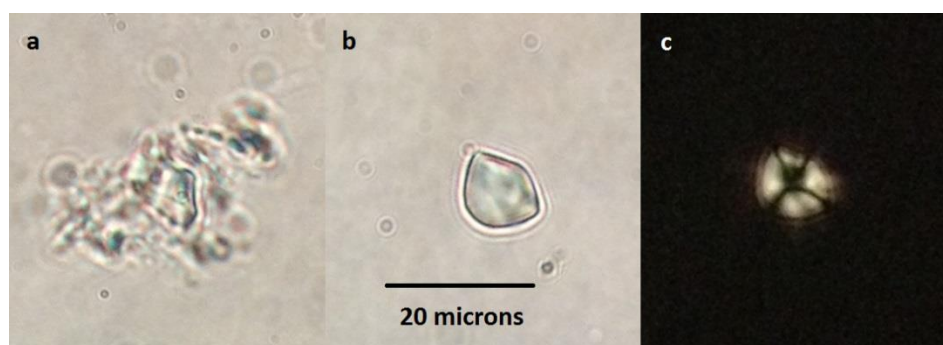


Figure E-12. Starch and phytolith particles recovered from calculus sample associated with Lokomotiv Burial R-15-1, scale applies to all pictures: (a) Possible phytolith embedded in demineralized calculus; (b,c) Starch grain under normal and cross polarized light respectively.



Figure E-13. Possible rod shaped diatom recovered from calculus associated with Lokomotiv Burial R-15-1.

#### L-9-1-1

The subsample from Lokomotiv Burial L-9-1-1, an adult female under 20 years of age, contained two phytoliths and one possible phytolith. One phytolith was trapezoidal in shape, 12.1  $\mu\text{m}$  in length, and could be rotated under the coverslip to confirm shape (Figure E-14a). The other was shaped somewhat like a conical flask, with an inclusion or void at one end (Figure E-14b). The opposite end may have had an inclusion as well. It was 12.9  $\mu\text{m}$  in size. The last possible phytolith was also trapezoidal in shape, 10.7  $\mu\text{m}$  in size, and exhibited a double border (see Chapter 3, Section 3.2.5; Figure E-14c). It would not rotate completely to confirm three-dimensional shape.

Four starch grains were also present. A large convex-lenticular starch grain was observed, measuring 25.5  $\mu\text{m}$ , with four to five concentric lamellae visible (Figure E-14d). The arms of the extinction cross were wide and weakly visible and the hilum was centrally placed (Figure E-14e, f). The extinction cross became a straight bar across the middle of the grain when it was rotated sideways (Figure E-14f). There was a slight protrusion to one end of the grain. This grain was distinct from reference contaminants and considered to have been incorporated during the life of the individual. The remaining starch grains could not be rotated during analysis and therefore the three-dimensional shapes could not be confirmed for any of them. They shared similar characteristics and may have come from the same source. A very small starch grain exhibited faint birefringence and an H-shaped extinction cross (Figure E-14g, h). An indentation/depression occurred at the centrally placed hilum. Another starch grain was lodged within demineralized calculus material and was also very small. It had a weak but observable H-shaped extinction cross and a slight depression at a centrally placed hilum (Figure E-14i, j). The last one had a slight depression at the central hilum and a slightly H-shaped extinction cross (Figure E-14k, l). These small grains measured 4.6, 5.6, and 9.5  $\mu\text{m}$  respectively. All were distinct from controls despite their association with a contaminated blank control (round 14). Still, they should be regarded cautiously.

Microbial activity was noted in the sample in the form of small opaque cells, likely bacteria. A small amount of lacquer was also present (see Figure 5.3b). A large brown particle may have been a fungal spore, with hyphae attached. An unknown opaque particle was likely mineral in origin (Figure E-15a). Lastly, a fragment of an unknown possible plant particle was also recorded (Figure E-15b).

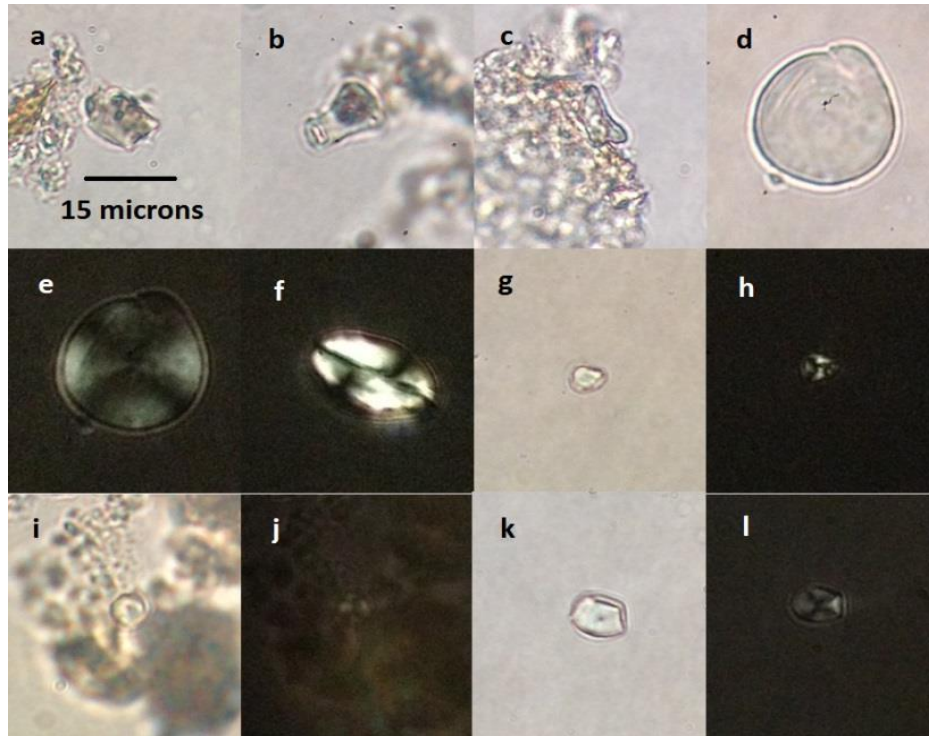


Figure E-14. Microparticles recovered from calculus associated with Lokomotiv Burial L-9-1-1, scale applies to all pictures: (a-c) Various possible phytoliths; (d-f) Large lenticular starch grain at different angles under normal and cross polarized light; (g-l) Various small starch grains under normal and cross polarized light; none could be rotated.

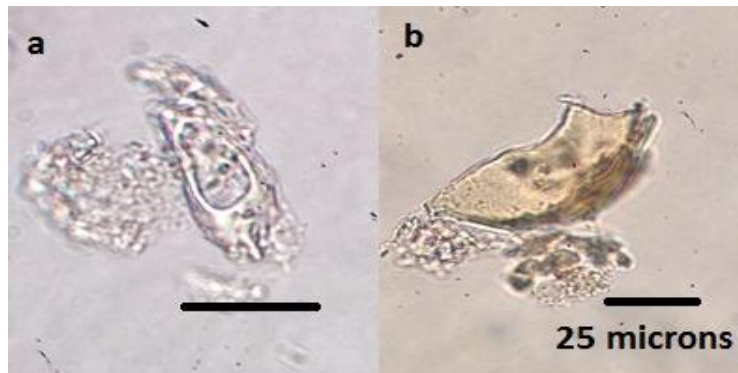


Figure E-15. Unknown particles recovered from calculus associated with Lokomotiv Burial L-9-1-1, scale in both pictures is 25  $\mu\text{m}$ : (a) Unknown opaque particle; (b) Possible plant particle fragment.

47-1

Shamanka II Burial 47-1 was an adult female, aged 20-25. Two samples were collected: one from the mesial surface of the right central mandibular incisor (sample one), and one from the lingual surface of the left first mandibular molar (sample two). Sample one contained one damaged starch grain, measuring 13.7  $\mu\text{m}$ , (Figure E-16a-c). It was concave-lenticular to discoid in shape. A round depression in the middle of the grain gave it the appearance of having double rings. Under cross polarized light, the central depression had no birefringence and the wide arms of the extinction cross were only seen on the outer edges of the body (Figure E-16b). It likely had a central hilum. A starch grain found within the mandible control swab for this individual had a similar overall shape, though birefringence was stronger in the control starch grain and the arms of the extinction cross were sharper (Figure E-16d, e). A starch grain observed in a bag swab for this individual also appeared to have almost identical damage to its surface (Figure E-16f). Due to the similarities the starch grain shared with contaminants it was considered a possible contaminant and eliminated from further consideration. Fungal activity in the form of large spores and hyphae was noted.

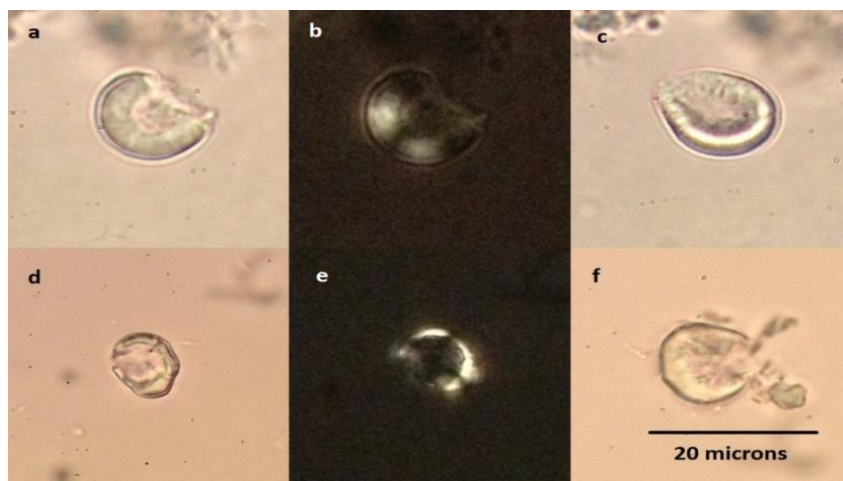


Figure E-16. Comparison between starch grain recovered from calculus associated with Shamanka II Burial 47-1, sample one and contaminants, scale applies to all pictures: (a-c) Starch grain, under normal and cross polarized light, from different angles; (d,e) Similar starch grain observed from mandible swab control; (f) Similar starch grain observed in bag swab control.

Sample two contained three starch grains. Two were damaged. One, measuring 13.2 $\mu$ m, appeared to have scalloped edges. It could only be seen from a side view and could not be moved to view at another angle, (Figure E-17a). Under cross polarized light, only two arms of the extinction cross were seen due to the damage sustained to the grain (Figure E-17b). No other characteristics could be determined for this starch grain. The other damaged starch grain also could not be rotated but appeared to be roundish-oval in shape with some scalloping along the edge of the grain. It was 12.5  $\mu$ m in length (Figure E-17c). Extinction arms appeared under cross polarized light though it was not clear if they formed an actual cross (Figure E-17d). This was again likely due to the damage sustained. Lastly, a tiny starch grain, about 5.5  $\mu$ m in length and with strong birefringence, was discovered attached to a piece of demineralized calculus (Figure E-17e, f). It had a central hilum, but no other features were distinguishable. It was kept in the study. Four to five spores, each between about 20-30  $\mu$ m in diameter, were also seen in the sample (Figure E-18). The spores were similar in appearance to *Lycopodium* spores but darker in color. Each appeared firmly attached to remaining demineralized calculus.

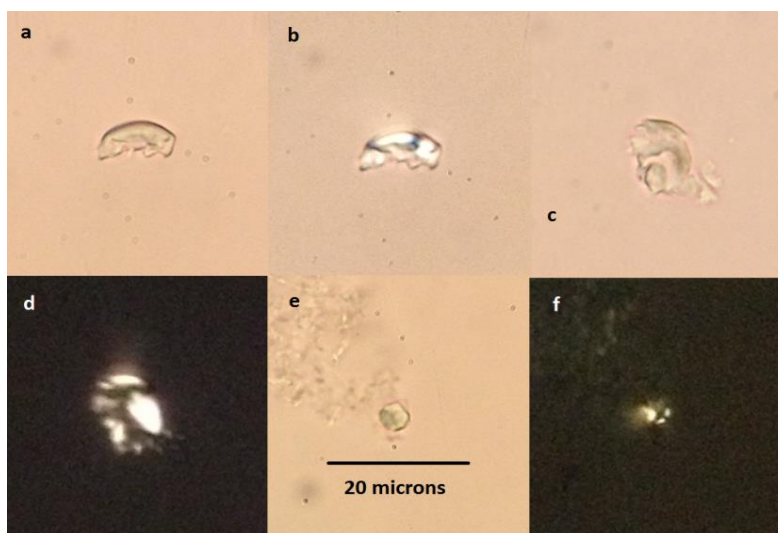


Figure E-17. Starch grains recovered from calculus associated with Shamanka II Burial 47-1, sample two, scale applies to all pictures: (a,b) Damaged grain with scalloped edges under normal and cross polarized light respectively; (c,d) Damaged roundish-oval shaped starch grain under normal and cross polarized light respectively; (e,f) Small starch grain associated with calculus material under normal and cross polarized light respectively.



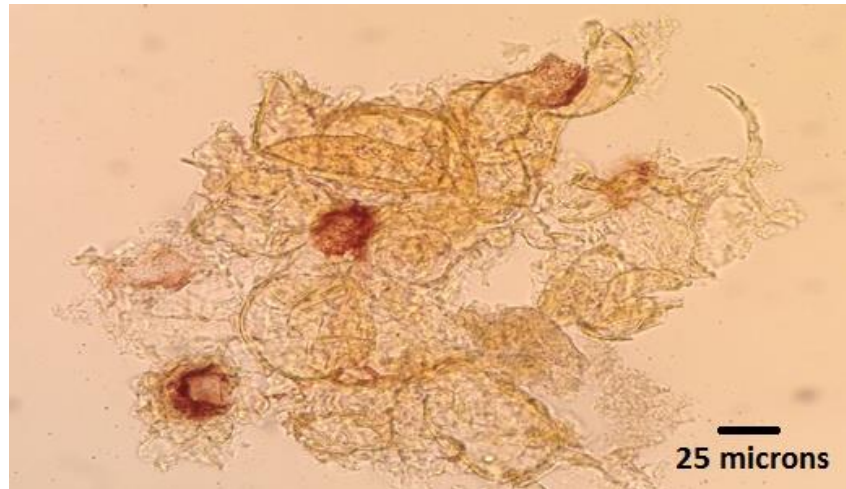


Figure E-18. Spores recovered from calculus associated with Shamanka II Burial 47-1, sample two.

#### 60-2

This sample originated from the lingual surface of the right mandibular first premolar of Shamanka II Burial 60-2, an adult female, aged 40-44. Two starch grains and one possible phytolith were present within the subsample. The researcher was unable to rotate one of the starch grains, and it broke into two pieces when the coverslip was pressed (Figure E-19a). It measured 13.2  $\mu\text{m}$  in length. The surfaces formed by the crack suggest it broke along a central fissure. It had a central hilum and sharp extinction cross arms under cross polarized light (Figure E-19b). Because it could not be rotated, a three-dimensional shape could not be confirmed though it appeared polyhedral in shape. It is considered to have been incorporated in calculus in vivo (see Chapter 5, Section 5.2.2). The other starch grain was smaller in size, measuring 8  $\mu\text{m}$ . It was round in shape, with a central hilum and a Y-shaped fissure or depression at the hilum (Figure E-19c, d). While this starch grain did not match controls exactly and was not deemed a contaminant, it should be regarded cautiously since its size and Y-shaped fissure were consistent with maize, though the polyhedral shape typical of maize was absent (Boyd et al. 2006). Originally, a possible “chef’s hat” shaped phytolith was identified (Figure E-19e). It could not be rotated to look at its three-dimensional shape and likely was a calculus-derived mineral grain; therefore it was not included in the phytolith analysis.

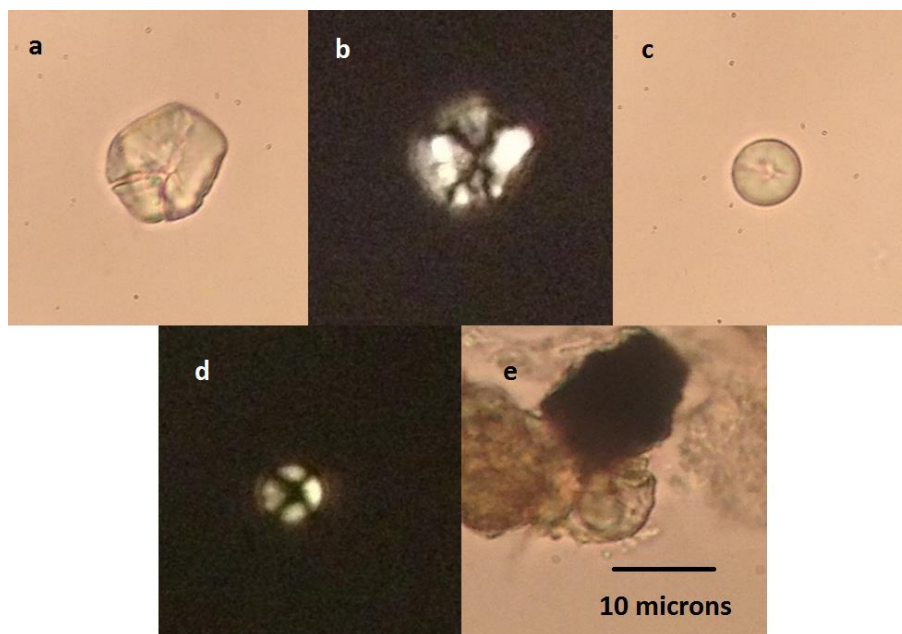


Figure E-19. Plant particles recovered from calculus associated with Shamanka II Burial 60-2, scale applies to all pictures: (a,b) Cracked starch grain under normal and cross polarized light respectively; (c,d) Small round starch grain under normal and cross polarized light respectively; (e) Possible phytolith, but more likely a calculus-derived mineral grain.

### 30-1

Shamanka II Burial 30-1 was an adult male, aged 35-50. The sample taken from the buccal surface of the right mandibular first molar of this individual was very large (see Figure 3.1d) and was therefore subdivided for analysis, with part reserved for stable isotopic analysis in future. The remaining portion was split into two samples, processed separately. The first (sample one) was left in HCl for 24 hours and the second (sample two) was left in for only 3 hours.

In the subsample from sample one, one starch grain was found attached to a small piece of demineralized calculus (Figure E-20a); the grain measured 16.8 $\mu$ m. It could be moved enough to get an idea of its shape, but could not be fully rotated. It appeared to be semi-circular in shape, but the base was slightly pointed where the hilum was located. The hilum was centrally placed on the base of the grain but it was not clear if it was centrally placed with regards to the grain as a whole. The arms of the extinction cross under cross polarized light were sharp (Figure E-20b). It was distinct from all contaminant starch grains observed. Fungal activity, in the form of a large spore-like particle with a hyphae stem attached, was noted. A few thin translucent sheets, possibly organic tissue of some type, were observed (Figure E-21). They were observed in both



subsamples analyzed from this individual, but not in subsamples from any other individual analyzed.

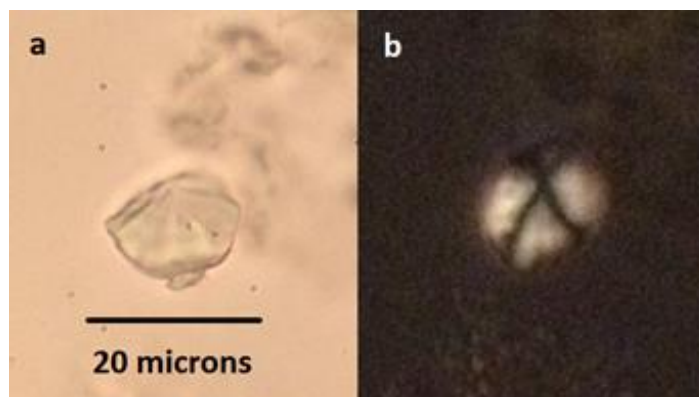


Figure E-20. Semi-circular starch grain recovered from calculus associated with Shamanka II Burial 30-1, sample one, scale applies to both pictures: (a) Starch grain under normal light; (b) Starch grain under cross polarized light.

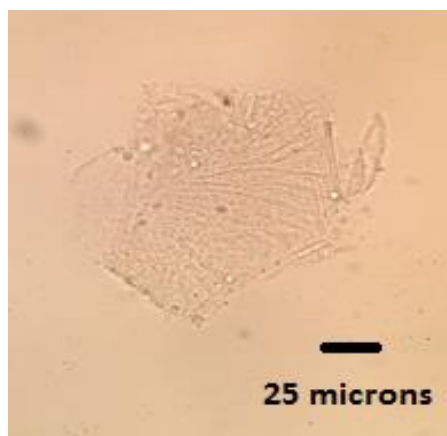


Figure E-21. Translucent unknown tissue-like material recovered from calculus associated with Shamanka II Burial 30-1, sample one.

The subsample from sample two contained two starch grains. The first starch grain was attached to demineralized calculus and could not be rotated, but appeared to be round to oval in shape. An X- or Y-shaped fissure was visible and the grain displayed a central hilum (Figure E-22a). The entire particle measured 9.3  $\mu\text{m}$ . The extinction cross arms were moderately sharp but birefringence was weak and inconsistent, indicating damage (Figure E-22b). This starch grain was distinct from contaminants. The second starch grain was round, 10  $\mu\text{m}$  in length, with

visible concentric lamellae and a central hilum (Figure E-22c). The arms of the extinction cross arms were quite sharp, but birefringence was weak and inconsistent along part of the grain, suggesting damage (Figure E-22d). Lenticular starch grains were found in controls, but their extinction crosses did not match this example, so it was kept in the analysis; still, this starch grain should be considered cautiously.

Another particle was positively identified as a test from a testate amoeba (Jacques Brochier, personal communication 2014; Figure E-22e, f; see Section 5.2.5). An unknown particle was opaque in color and had a thick concave-lenticular shape when rotated (Figure E-22g). It had no birefringence. Stuart (personal communication 2014) speculated that it may have been a much corroded *Equisetum* (field horsetail) pollen grain. Conifer pollen was also recorded (Figure E-23).

One rod-shaped phytolith was observed but could not be rotated. It was still tightly attached to demineralized calculus (Figure E-22h). One end of the phytolith was obscured, but the particle was roughly 73  $\mu\text{m}$  in length. Fibrous fungal hyphae were embedded in some demineralized calculus pieces, and an unknown hair-like particle was noted. The hair-like particle had a pinkish hue to it, with a thick base compared to the tip.

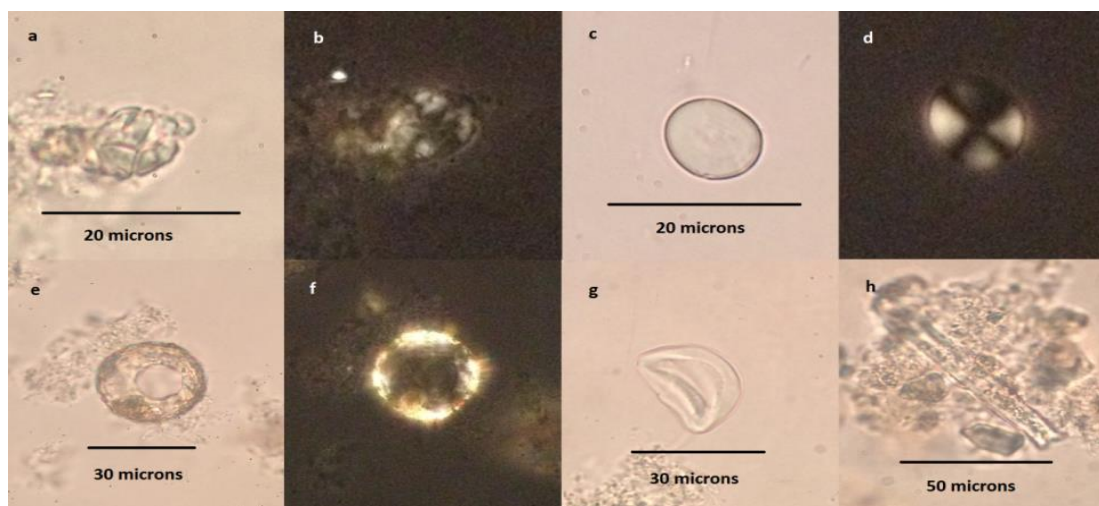


Figure E-22. Microparticles recovered from calculus associated with Shamanka II Burial 30-1, sample two: (a, b) Possibly damaged oval starch grain attached to calculus under normal and cross polarized light respectively; (c,d) Possibly damaged lenticular starch grain under normal and cross polarized light respectively; (e,f) Testate amoeba test under normal and cross polarized light respectively; (g) Possible highly eroded *Equisetum* (field horsetail) pollen grain; (h) Rod-shaped phytolith embedded in calculus material.

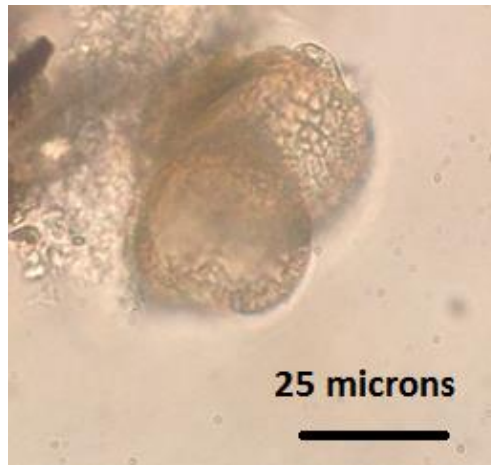


Figure E-23. Conifer pollen grain recovered from calculus associated with Shamanka II Burial 30-1, sample two.

#### 46-1

Two samples were taken from Shamanka II Burial 46-1, an adult male, aged 25-29. Sample one was taken from the buccal surface of the right second maxillary premolar. A few potential and definite starch grains were observed within the subsample. A large roundish-oval starch grain, measuring 24  $\mu\text{m}$ , displayed concentric lamellae (five to six rings) and an extinction cross with wide arms at the edge of the grain and narrowing toward the center (Figure E-24a, b). It had a central hilum and cracking along the edges, indicative of damage. Another starch grain also exhibited damage (Figure E-24c, d). The potential extinction cross was very weak and cross arms were very wide. The grain was likely roundish in shape but had a serrated portion where it appeared that part of the original grain had been torn off. There may have been a slight depression in the center of the starch grain, but no other characteristics could be determined due to the damage. The grain measured 11.5  $\mu\text{m}$ . Both starch grains were considered to be non-contaminants. One other particle was noted. It consisted of a central round mass with two triangular protrusions on either side (Figure E-25a). It was birefringent, but exhibited no extinction cross. Lastly, a large clear contaminant fiber was found, raising caution with regards to the subsample.

Sample two was taken from the lingual surface of the right first mandibular molar. Only one starch grain was found within the subsample and no phytoliths. The starch grain was similar to the starch grains seen in Lokomotiv Burials L-15-1-1 and L-20-2-1. It was very small and had a dehydrated-looking texture to its surface, with only a faint partial extinction cross. It could not

be rotated to more fully observe the starch grain. This example was eliminated as a possible contaminant. Fungal activity was noted, in the form of large quantities of hyphae throughout the subsample. Large clumps of brown particulates were observed, but indiscernible particle boundaries made it difficult to distinguish if these were fungal or pollen aggregates. Two brown circular particles were identified tentatively as pollen grains, though they appeared highly degraded (Glenn Stuart, personal communication 2014; Figure E-25b, c).

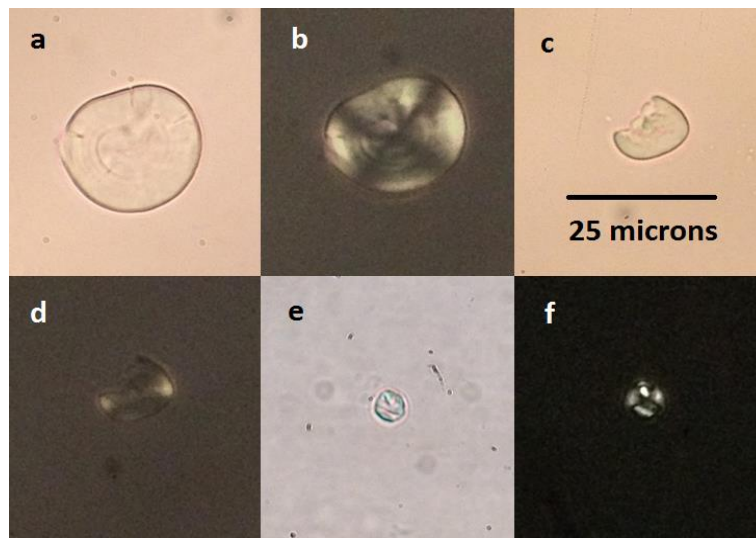


Figure E-24. Starch grains recovered from calculus associated with Shamanka II Burial 46-1, scale applies to all pictures: (a,b) Large damaged starch grain with visible lamellae, from sample one, under normal and cross polarized light respectively; (c,d) Damaged starch grain, from sample one, under normal and cross polarized light respectively; (e,f) Possible contaminant starch grain, from sample two, under normal and cross polarized light respectively.



Figure E-25. Other particles found in calculus associated with Shamanka II Burial 46-1, scale in all pictures is 25  $\mu$ m: (a) Unknown mineral-like particle with protrusions, from sample one; (b,c) Possible degraded pollen grains, from sample two.

Shamanka II Burial 24 was an adult male, aged 25-35. The sample was taken from the buccal and distal portion of the right maxillary third molar, and a subsample contained one roundish starch grain, measuring 16.5  $\mu\text{m}$  in diameter (Figure E-26a, b). It was attached to calculus material within the sample and had a slightly granular surface and a darker gray colour than many of the other starch grains. Birefringence was quite strong and the extinction cross arms were quite sharp. There was a small indentation at the centrally placed hilum. It was considered to have been incorporated into calculus in vivo.

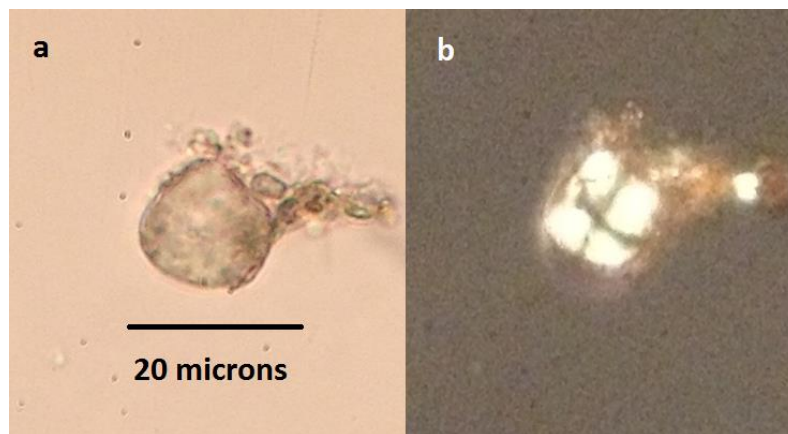


Figure E-26. Starch grain recovered from calculus associated with Shamanka II Burial 24, scale applies to both pictures: (a) Starch grain under normal light; (b) Starch grain under cross polarized light.

#### 76-1

A sample was taken from the buccal surface of the right mandibular canine from Shamanka II Burial 76-1, an adult male, aged 40-50, and one possible starch grain was observed in the subsample (Figure E-27a). The starch grain did not rotate under the coverslip, and therefore the three-dimensional shape could not be determined. Only two arms of a possible extinction cross were visible (Figure E-27b). It did not appear to match identified contaminants and was therefore kept in the analysis. Opaque fungal hyphae were noted. One possible contaminant cotton fiber was also recorded.

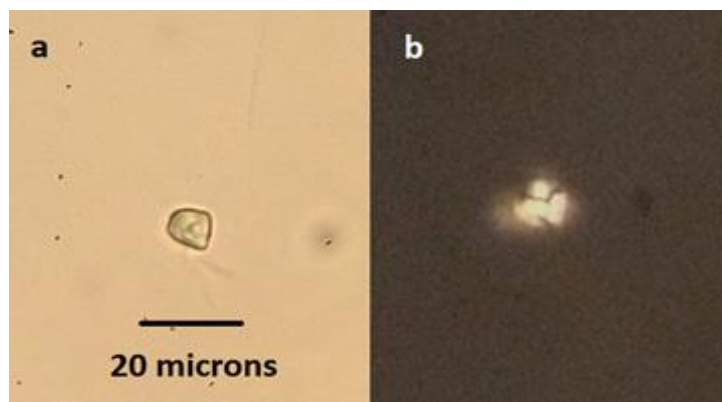


Figure E-27. Possible starch grain recovered from calculus associated with Shamanka II Burial 76-1, scale applies to both pictures: (a) Starch grain under normal light; (b) Starch grain under cross polarized light.

#### 66-1

The sample was taken from the buccal surface of the right mandibular central incisor of Shamanka II Burial 66-1, an adult female, aged 25-35, and a subsample contained two starch grains. The first was roundish to polyhedral in shape with a simple straight fissure through the body of the grain (Figure E-28a). The entire grain measured 13.4  $\mu\text{m}$  in length. The hilum was slightly acentric, and the arms of the extinction cross were sharp (Figure E-28b). This starch grain was similar to some contaminant starch grains, but the placement of its hilum did not match; therefore, it was not removed as a contaminant but should be regarded cautiously. The other starch grain was oval in shape, with a central depression or stellate fissure on the surface. This grain measured 22  $\mu\text{m}$  (Figure E-28c). Under cross polarized light, a central hilum was seen, and the arms of the extinction cross were sharp (Figure E-28d). Visually, it was an almost identical shape and birefringence pattern to a starch grain noted in blank control 12 (Appendix D, Figure D-10c; Figure 5.1). It was therefore considered a contaminant. Another possible starch grain was noted when originally analyzing the sample, but, upon further investigation, it was determined that it did not exhibit a true extinction cross, and it was discarded from the analysis. A large number of fungal hyphae were observed within the sample, and a large spore, possibly associated with the fungal activity, was stuck on the edge of some demineralized calculus (Figure E-29).



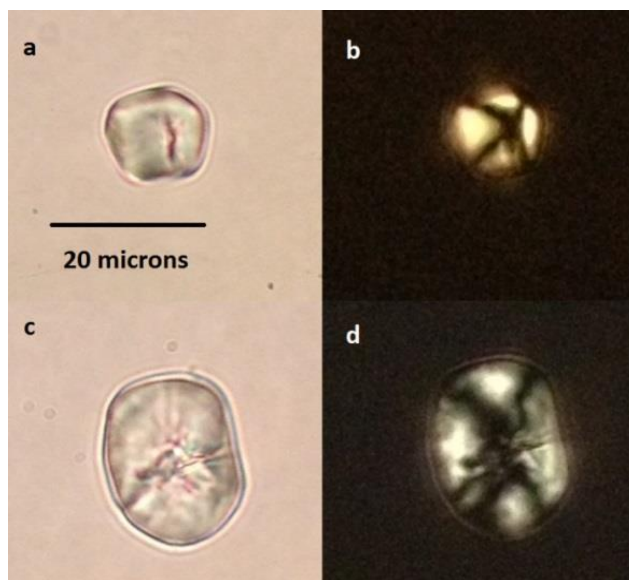


Figure E-28. Starch grains recovered from calculus associated with Shamanka II Burial 66-1, scale applies to all pictures: (a,b) Roundish-polyhedral grain with straight fissure, under normal and cross polarized light respectively; (c,d) Oval starch grain with depression or stellate fissure under normal and cross polarized light respectively.



Figure E-29. Spore, likely associated with fungal activity, recovered from calculus associated with Shamanka II Burial 66-1.

### 26-3

Shamanka II Burial 26-3 was a juvenile, aged 6-8, of undetermined sex. The lingual surface of the right deciduous first molar was sampled, and a subsample contained two starch grains. One was 8.2  $\mu\text{m}$  in length, and could not be rotated to view three-dimensional shape. In two-dimensional view, it had a trapezoidal shape with a depression on the surface (Figure E-

30a). It appeared to have had an acentric hilum but this could not be confirmed without rotating (Figure E-30b). The arms of the extinction cross were quite sharp. The second starch grain was round with a stellate-shaped crack or depression at the central hilum (Figure E-30c). It was 9.8  $\mu\text{m}$  in diameter. The birefringence was quite weak under cross polarized light, but a wide-armed cross was clearly present (Figure E-30d). Both were considered non-contaminants. Five other particles were originally noted as possible starch grains but due to a lack of structure or extinction cross they have been disregarded for the analysis. Fungal activity was noted in the form of opaque hyphae.

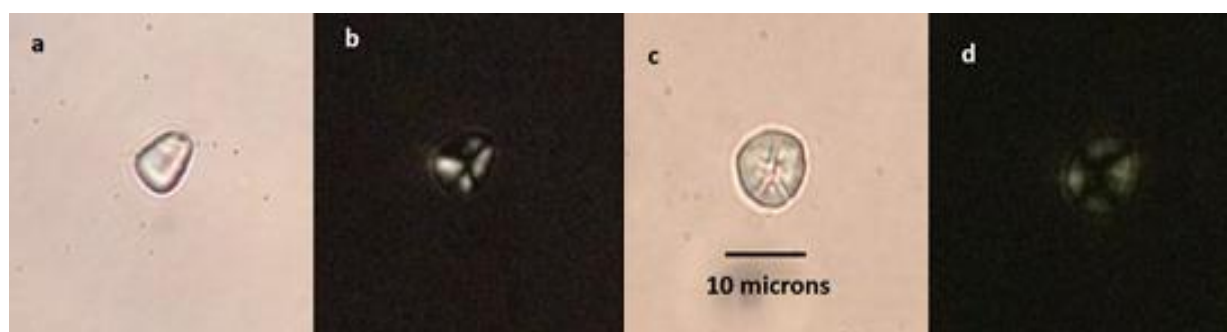


Figure E-30. Starch grains recovered from calculus associated with Shamanka II Burial 26-3, scale applies to all pictures: (a,b) Trapezoidal starch grain under normal and cross polarized light respectively; (c,d) Round starch grain with stellate fissure/depression under normal and cross polarized light respectively.

#### 109-1

No starch grains or phytoliths were found within the subsample from Shamanka II Burial 109-1, a female, aged 40-50. The sample was taken from the buccal surface of the left maxillary first molar and the subsample was dominated by brown fungal spore structures with darker brown borders and lighter brown, translucent bodies. Many of these spores were attached to hyphae (e.g., see Figure 5.9g). Two starburst particles were present and identified as possible spores (Figure E-31a, b). A similar particle was found in a bag control from Ust'-Ida I Burial 44-2 and therefore the particles were deemed likely to be contaminant fungal residue (see Chapter 5, Section 5.2.4 for more information on the origins of fungal spores and hyphae; Appendix D, Figure D-8d). The body of each was round, and spicules or hairs extended out from the body, giving it its starburst shape. The spicules or hairs were longer in one of the particles than the other.



Another particle may be a fragment of a diatom, but this is quite speculative (Figure E-31c).

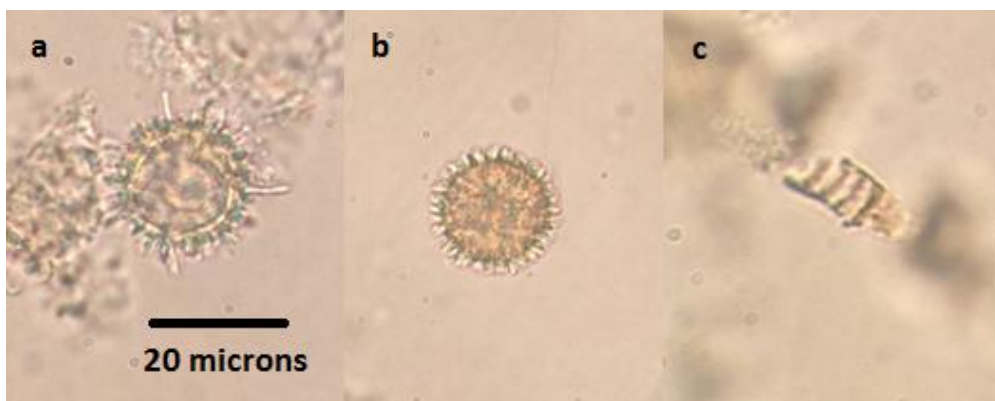


Figure E-31. Microparticles recovered from calculus associated with Shamanka II Burial 109-1, scale applies to all pictures: (a,b) Spicule-covered spores likely associated with mold/fungal activity; (c) Possible diatom with ribbing or ornamentation.

## 67

The sample from Shamanka II Burial 67, a juvenile, aged 7-9, was taken from the lingual surface of the left deciduous molar and, at 0.6 mg, was the smallest sample taken from Shamanka II. The subsample contained two starch grains. One was roundish in shape, with three deep fissures, separating the grain into three lobes (Figure E-32a-d). One fissure was seen to run the entire length of the starch grain (Figure E-32a). The grain was 8.8  $\mu\text{m}$  in length. The other starch grain was discoid to convex-lenticular in shape, with faint concentric lamellae visible and a protrusion at one end (Figure E-32e). This grain was 11.9  $\mu\text{m}$  and had a central hilum. The arms of the extinction cross were sharp (Figure E-32f). Clear organic fibers were seen in the sample, suggesting a small amount of contamination, but both starch grains were quite distinct from those in controls and were therefore considered non-contaminants.

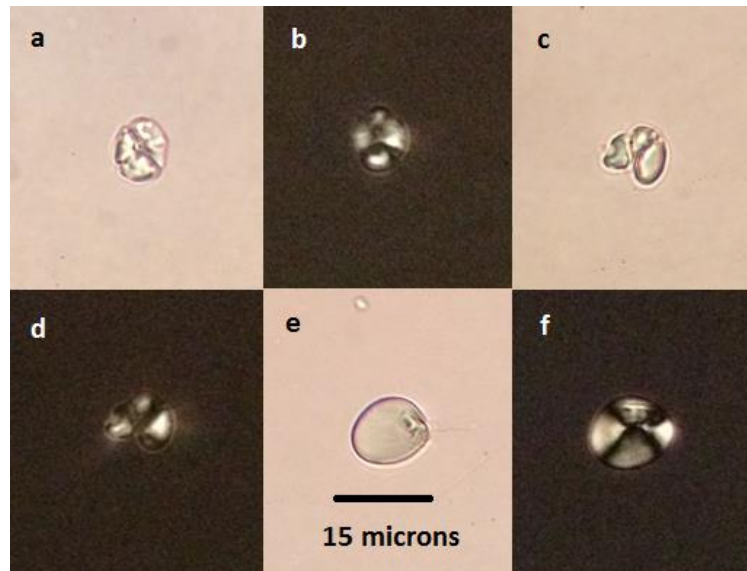


Figure E-32. Starch grains recovered from calculus associated with Shamanka II Burial 67, scale applies to all pictures: (a,c) Three lobed starch grain at different angles under normal light; (b,d) Three lobed starch grain at different angles under cross polarized light; (e,f) Discoid to lenticular shaped starch grain under normal and cross polarized light respectively.

#### *Ust'-Ida I Burials*

##### 14-1-1

Ust'-Ida I Burial 14-1-1 was a juvenile male, aged 18-20. The distal interproximal surface of the right mandibular central incisor was sampled for analysis. No particles of interest were observed within the subsample.

##### 6-1-1

Ust'-Ida I Burial 6-1-1 was an adult male, aged 35-50. Calculus from the distal interproximal surface of the right maxillary second premolar was analyzed. Two starch grains were observed within a subsample. Both were large starch grains (33 and 35.9  $\mu\text{m}$  respectively) with visible concentric lamellae; both were damaged. One was roughly convex-lenticular in shape and had considerable cracking on its surface (Figure E-33a). It had no birefringence, potentially due to the damage it sustained. The other could not be rotated and it was round in two-dimensional view (Figure E-33b). There was cracking around the edges of the starch grain, and birefringence was very weak and inconsistent, indicating damage (Figure E-33b, c). While

both were considered to be distinct from contaminants, a large, rigid, contaminant pink/red fiber was also found within the sample (see Figure 5.2f); therefore the starch grains should be regarded cautiously.



Figure E-33. Starch grains recovered from calculus associated with Ust'-Ida I Burial 6-1-1, scale applies to all pictures: (a) Damaged starch grain with cracking, no birefringence; (b,c) Large starch grain with weak birefringence under normal and cross polarized light respectively.

### 38-1-1

Ust'-Ida I Burial 38-1-1 was an adult male, aged 35-45, and the mesial interproximal surface of the left mandibular lateral incisor was sampled. A subsample contained one damaged starch grain and another possible starch grain. The former was represented by only half of the original grain, measuring 12.2  $\mu\text{m}$  (Figure E-34a, b). Due to the damage and because the grain could not be rotated, the shape could not be determined. It was distinct from controls and was therefore considered a non-contaminant. The latter had an irregular polyhedral shape, and birefringence was very weak. No distinct extinction cross was observed. A very similar particle was observed in a blank control from the round (round nine) following the round associated with this particle (Appendix D, Figure D-10m). It was therefore discarded from analysis as a potential contaminant. Possible fungal spores and hyphae were noted, as well as a tricolporate pollen grain, possibly from the Primulaceae or Rhamnaceae family (Glenn Stuart, personal communication 2014; Figure E-35). One rigid clear fiber was likely synthetic in origin and indicated contamination at some step in the process.

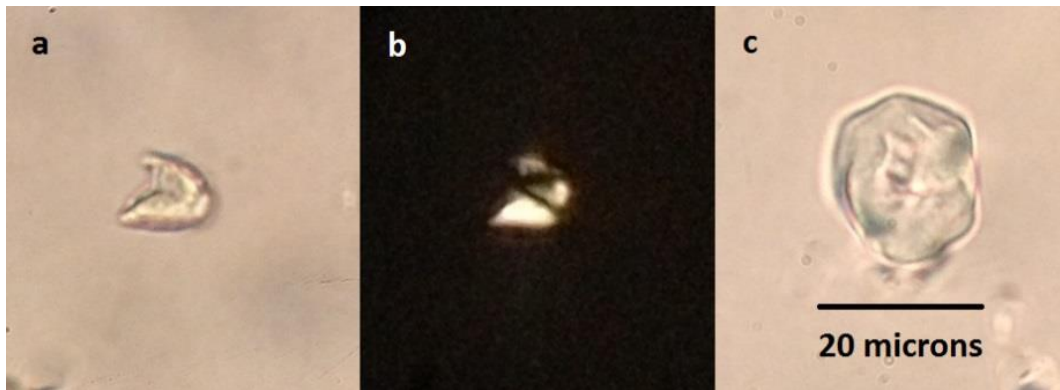


Figure E-34. Starch grains recovered from calculus associated with Ust'-Ida I Burial 38-1-1, scale applies to all pictures: (a,b) Damaged starch grain under normal and cross polarized light respectively; (c) Possible starch grain with only very weak birefringence.



Figure E-35. Tricolporate pollen grain recovered from calculus associated with Ust'-Ida I 38-1-1.

#### 45-1-1

Ust'-Ida I 45-1-1 was an adult male, aged 22-30. The sample was taken from the mesial interproximal surface of the left mandibular canine. Small amounts of fungal hyphae and a few organic fibers were found adhering to demineralized calculus within the subsample. One starch grain was recorded, measuring 22  $\mu\text{m}$  (Figure E-36a, b). It was damaged, with cracks visible on the surface. The extinction cross arms were moderate to wide. It was roundish-oval in shape, and the central hilum was visibly outlined on the grain. A starch grain with a visible outline around the hilum was also found in the blank control associated with this sample, but was much smaller than the starch grain seen here. Therefore, the starch grain from this subsample was not considered a contaminant but should be regarded cautiously.

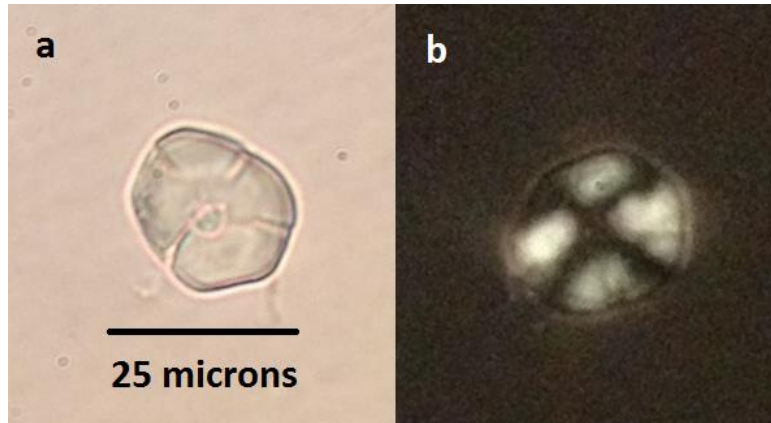


Figure E-36. Starch grain with visible cracking recovered from calculus associated with Ust'-Ida I Burial 45-1-1, scale applies to both pictures: (a) Starch grain under normal light; (b) Starch grain under cross polarized light.

### 33-1-1

Ust'-Ida I Burial 33-1-1 was a juvenile of undetermined sex, aged 12-15. Calculus was taken from the buccal surface of the left maxillary second premolar. A unique starch grain was found within the subsample. It was discoid in shape with a small, deep depression in the center (Figure E-37a-c). It was 17.4  $\mu\text{m}$  in size. The arms of the extinction cross were seen on the edges of the starch grain but not in the middle where the depression was. It was unlike anything seen in the controls and was therefore deemed not a contaminant. One blue and one clear fiber were found within this sample as well.



Figure E-37. Starch grain within calculus associated with Ust'-Ida I Burial 33-1-1, scale applies to all pictures: (a,b) Starch grain from different angles, showing distinct shape under normal light; (c) Same grain under cross polarized light.

#### 44-2

Ust'-Ida I Burial 44-2 was a juvenile of undetermined sex, aged 5-6, and the lingual surface of the deciduous right molar was sampled. Nothing of interest was found within the subsample from this individual. The entire sample was quite small, weighing 0.6 mg.

#### 19-1

Ust'-Ida I Burial 19-1 was an adult male, aged 30-35. Calculus was analyzed from the lingual surface of the right mandibular second molar. Nothing of interest was found within this subsample. This entire sample was also small, weighing 0.8 mg.

#### 5-1-1

Ust'-Ida I Burial 5-1-1 was a juvenile of undetermined sex, aged 7-9; a sample was taken from the mesial surface of the right mandibular lateral incisor. Large masses of solid, transparent to opaque film fragments were observed in the subsample. This was identified as likely representing lacquer, although no lacquer was noted on this tooth surface during sampling. Much of the calculus in the subsample remained undissolved and adherent to this lacquer material, despite treatment of the sample with HCl (see Figure 5.3c, d). Nothing else of interest was observed.

#### 56-1

Ust'-Ida I Burial 56-1 was an adult male, aged 35-50. The sample was taken from the distal interproximal surface of the right maxillary second molar. Two starch grains and one possible grain were found within the subsample. One of the definite starch grains was oval with a circular depression or outline around the hilum and a slightly acentric hilum (Figure E-38a, b). It was 11.9  $\mu\text{m}$  in length. Some starch grains seen in controls had a similar circular depression and one, from a bag control from Shamanka II 46, had a similar shape and hilum position (Appendix D, Figure D-2a). This contaminant starch grain also exhibited a fissure, which was not seen in the starch from this sample. Still, it was eliminated from analysis as a precaution since the other characteristics were so close. Another of the two definite starch grains could not be moved but was likely oval to round, with one side coming to a point. This grain measured 9.3  $\mu\text{m}$ . The hilum was acentric and located opposite the side that came to a point (Figure E-38c, d). Birefringence



was moderate and the extinction cross arms were quite sharp. No control starch grains matched this grain, so it was kept in the analysis.

The possible starch grain was extremely small (less than 5  $\mu\text{m}$ ) and could barely be seen at 600X magnification. No features could be distinguished other than two possible arms of an extinction cross. It was quite a bit smaller than any starch grains observed in contaminant controls and was not considered a contaminant for this analysis. This may have been a transient starch grain. A possible pyramidal/triangular phytolith, 25  $\mu\text{m}$  in length, appeared attached to demineralized calculus, though it could not be rotated and identification was tentative (Figure E-39). One blue fiber was identified, indicating some contamination.

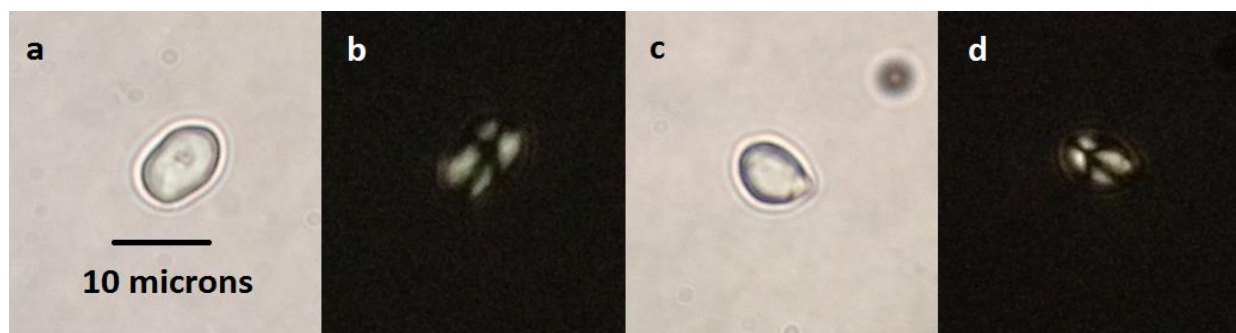


Figure E-38. Starch grains recovered from calculus associated with Ust'-Ida I Burial 56-1, scale applies to all pictures: (a,b) Oval starch grain with small depression at hilum under normal and cross polarized light respectively; (c,d) Starch grain with pointed end and acentric hilum seen under cross polarized light respectively.



Figure E-39 Possible phytolith recovered from calculus associated with Ust'-Ida I Burial 56-1.

### 30-1-1

Ust'-Ida I Burial 30-1-1 was an adult female, over 50 years of age. The buccal surface of the right mandibular second premolar was sampled. A number of particles were identified within the subsample. One oval starch grain, 19.8  $\mu\text{m}$  in diameter, was identified, with a rough uneven surface texture and a central hilum (Figure E-40a, b). The middle of the starch grain was distinct, with a slightly pinkish hue compared to the rest of the starch grain. A depression was not seen when the particle was rotated. Its shape and birefringence resembled some starch grains seen in controls and it was therefore considered a contaminant. Another possible tiny starch grain was found attached to demineralized calculus but could not be rotated. The surface of the starch grain looked shrunken or dehydrated and a net-like pattern was seen under cross polarized light rather than an extinction cross (Figure E-40c, d). Similar starch grains were seen in controls and this example was eliminated from analysis as a contaminant. Fungal hyphae and large spores were noted.

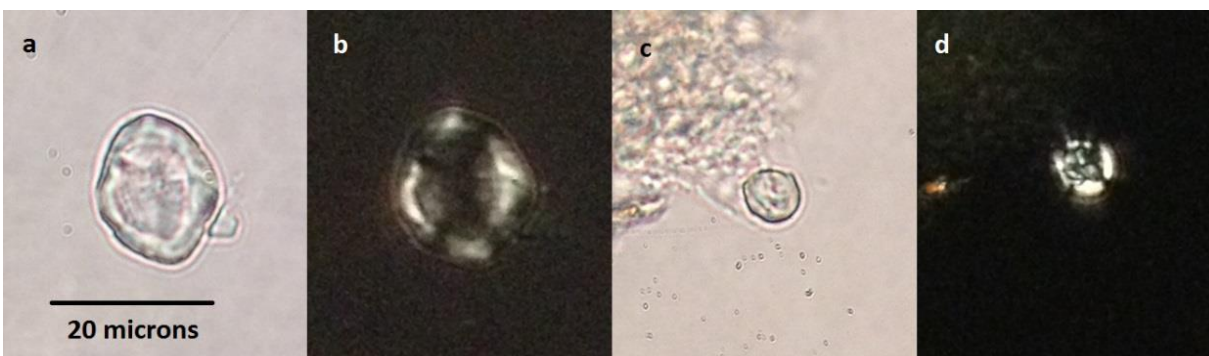


Figure E-40. Starch grains recovered from calculus associated with Ust'-Ida I Burial 30-1-1, scale applies to all pictures: (a,b) Oval starch grain with uneven surface texture under normal and cross polarized light respectively; (c,d) Small contaminant possible starch grain with dehydrated texture and only a possible extinction cross under normal and cross polarized light respectively.

### *Khuzhir-Nuge XIV Burials*

### 52

Khuzhir-Nuge XIV Burial 52 was an adult, aged 25-35, of undetermined sex. Two subsamples were taken from this individual, obtained from the buccal surface of the right mandibular second premolar. The second subsample yielded one distinct starch grain, unlike any



seen in controls and therefore considered to have been incorporated into calculus in vivo. It was roundish to oval in shape with a large circular outline in the center at the hilum (Figure E-41a). There was no birefringence within the central outline but birefringence was clearly seen about the exterior (Figure E-41b). The central outline did not appear to indicate a depression and the entire grain measured 18.3  $\mu\text{m}$ . One possible phytolith was found within subsample one. The phytolith measured 11.4  $\mu\text{m}$  (Figure 5.30c). It could not be moved to determine three-dimensional shape but was noted because of a visible inclusion or central void. Fungal activity was noted, in the form of hyphae and large spores, in both subsamples. A number of black to blue-black charcoal fragments were observed (see Chapter 5, Section 5.2.5).

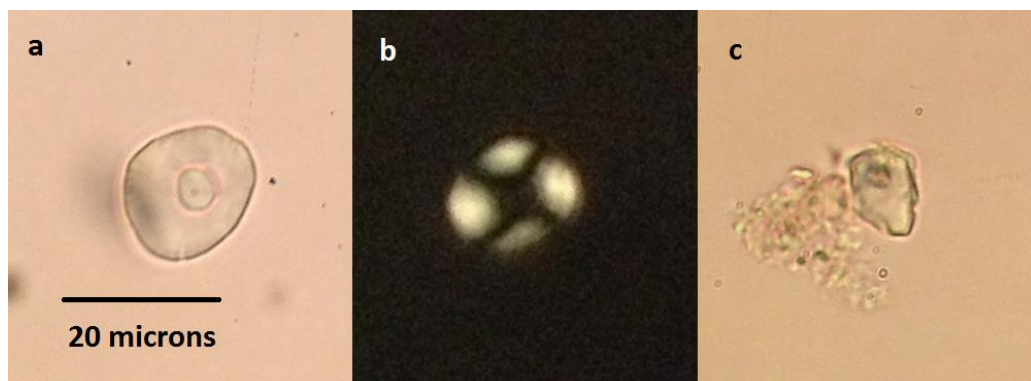


Figure E-41. Starch grain and phytolith recovered from calculus associated with Khuzhir-Nuge XIV Burial 52, scale applies to all pictures: (a,b) Distinct starch grain, from subsample two, with large circular outline at hilum under normal and cross polarized light respectively; (c) Possible phytolith, from subsample one, with inclusion or central void on surface.

## 27-1

Individual Burial 27-1 was an adult male, aged 35-50. Two subsamples were examined, taken from the buccal surface of the left maxillary first molar. Two possible phytoliths and one starch grain were found within subsample one. One phytolith was approximately 10  $\mu\text{m}$  in length and had a “chef’s hat” shape (Figure E-42a). Another lacked a prominent border but had a conic shape consistent with a cell type known as a trichome (Figure E-42b). It measured 8.8  $\mu\text{m}$ . The starch grain shattered into two pieces when it was pressed upon in an attempt to rotate it, similar to the starch grain from Shamanka II Burial 60-2. It was likely polyhedral in shape, with a

shallow fissure in the middle and central hilum (Figure E-42c, d). The entire grain measured 18  $\mu\text{m}$  and exhibited strong birefringence with sharp extinction cross arms. It was kept in the study.

Within subsample two, one to two possible phytoliths were noted. One was mostly cylindrical in shape, with voids observable from the surface (Figure E-42e). The other particle lacked a distinct structure and may not have been a phytolith at all. However, it was noted because of an inclusion or void visible (Figure E-42f). These possible phytoliths were attached to demineralized calculus and could not be rotated, in addition to exhibiting only a few characteristics of phytoliths; this was the basis for the tentative identification. Fungal activity was noted, in the form of brown hyphae and a few small brown spores, as well as some bacterial chains.

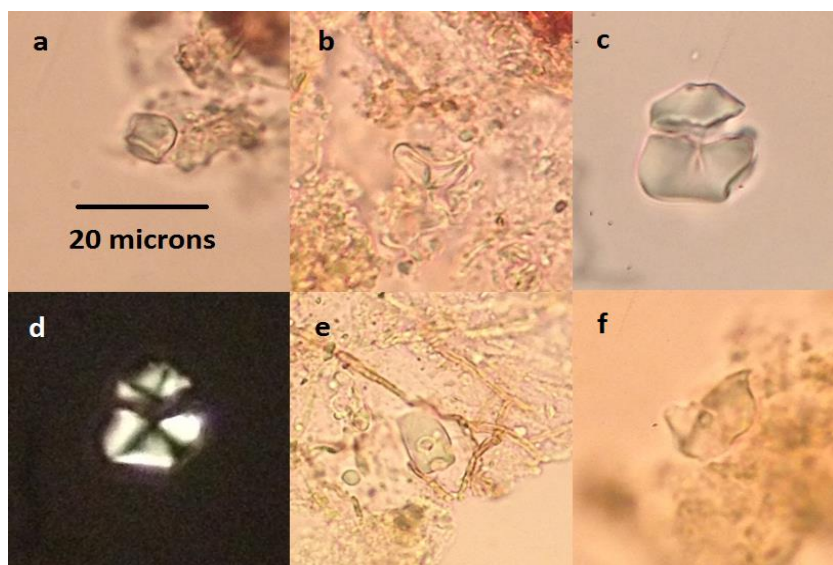


Figure E-42. Starch grains and phytoliths recovered from calculus associated with Khuzhir-Nuge XIV Burial 27-1, scale applies to all pictures: (a) “Chef’s hat” shaped possible phytolith, from subsample one; (b) Possible conical phytolith, from subsample one; (c,d) Shattered starch grain, from subsample one, under normal and cross polarized light respectively; (e) Cylindrical possible phytolith, from subsample two; (f) Possible phytolith, from subsample two, with little structure but visible void.

### 35-2

Khuzhir-Nuge XIV Burial 35-2 was a juvenile, aged 8-10, of an undetermined sex. Two subsamples were analyzed for this individual, taken from a sample removed from the buccal surface of the left permanent maxillary first molar. No starch grains or phytoliths were found

within either subsample. A small amount of hyphae and a number of black to blue-black charcoal particles were noted within subsample one (see Chapter 5, Section 5.2.5), as well as one unknown fragment that may have been a plant part of some type (Figure E-43). Subsample two yielded more black charcoal particles.



Figure E-43 Unknown possible plant fragment recovered from calculus from Khuzhir-Nuge XIV Burial 35-2, sample one.

### 37-2

Khuzhir-Nuge XIV Burial 37-2 was a juvenile of undetermined sex, aged between 14 and 17. A small deposit of only 0.6 mg was removed from the mesial interproximal surface of the right mandibular permanent mandibular central incisor. No starch grains were confidently identified within the subsample. A large opaque polyhedral particle, measuring 29.7  $\mu\text{m}$ , was identified and exhibited many of the visual properties of a starch grain but no birefringence (Figure E-44a, b). It did not appear to be damaged. It was very similar to the starch grains seen in Ust'-Ida I Burial 38-1-1 and Ust'-Ida I blank control round 9 (Appendix D, Figure D-10m), and was therefore considered a contaminant. Opaque to brownish hyphae and a few possible fungal aggregates were observed within the subsample.



Figure E-44. Contaminant particle with some starch characteristics recovered from calculus associated with Khuzhir-Nuge XIV Burial 37-2, scale applies to both pictures: (a) Front view under normal light; (b) Rotated view under normal light.

### 33

Khuzhir-Nuge XIV Burial 33 was a very young juvenile, aged 3-5, and the sample taken was very small, weighing only 0.2 mg. This was the youngest individual sampled. The sample came from the buccal surface of the deciduous right maxillary molar. No starch grains or phytoliths were found. A few fungal hyphae were noted among calculus material. Two large clumps of brown particulates were observed, but distinct boundaries were not distinguishable, making it impossible to determine if these were fungal or pollen aggregates.

### 12

Khuzhir-Nuge XIV Burial 12 was an adult, aged 25-35, of indeterminate sex. The sample, taken from the buccal surface of the right maxillary second premolar, was separated into two pieces because a fragment of the calculus fell into the researcher's hand while sampling.

Sample one was the calculus piece that had no contact with the researcher. The subsample from sample one contained three starch grains. Two of these were similar in shape. They were semi-spherical with central hila and strong, sharp extinction crosses. The larger of the two was 22  $\mu\text{m}$  in diameter (Figure E-45a, b). It appeared to be attached to the coverslip, with one end flush with the glass and unmovable, so it was considered a contaminant as a precaution. The second starch grain had a double-faceted bottom, causing a slight ridge along the surface (Figure E-45c, d). This one measured 16.8  $\mu\text{m}$  in diameter. A similar starch grain was found in a mandible control swab associated with Ust'-Ida I Burial 30-1-1, though the starch grain from the sample lacked the fissure seen in the control (Appendix D, Figure D-3d) and birefringence was different. Therefore this starch grain should be regarded with caution but was not considered a

contaminant here. The third starch grain was convex-lenticular in shape, measuring 24  $\mu\text{m}$ , with a central hilum (Figure E-45e). The arms of the extinction cross were wide and seemed to be more H- than X-shaped. The birefringence of the starch grain was inconsistent, indicating that the grain was likely damaged (Figure E-45f). It was distinct from contaminants and kept in the analysis. Fungal spores and hyphae were present.

Sample two was the piece that touched the researcher's hand. The subsample consisted of a similar array of particles but contained only one possible starch grain (Figure E-45g, h). It was very small ( $\sim 5\mu\text{m}$ ) and could not be rotated under the coverslip. Similar small, featureless starch grains were found in control swabs and it was therefore considered a contaminant as a precaution.

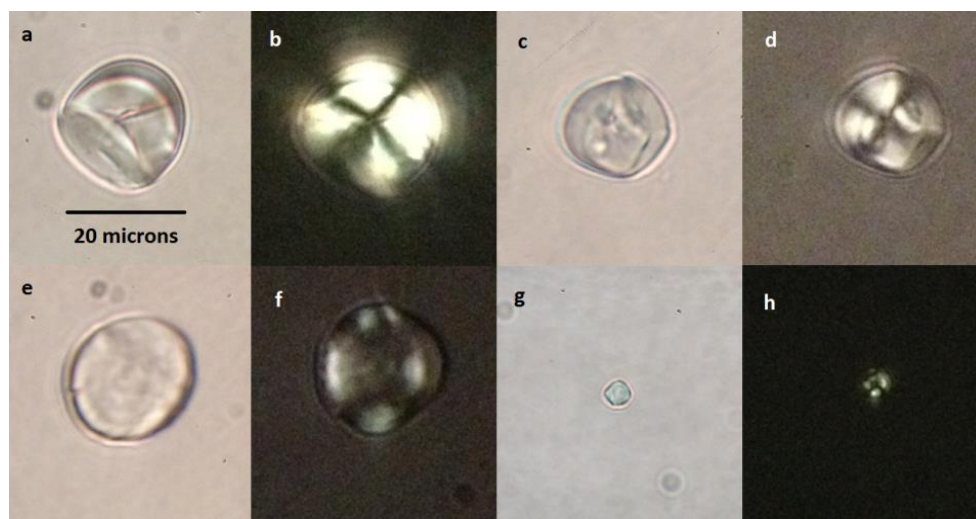


Figure E-45. Starch grains recovered from calculus associated with Khuzhir-Nuge XIV Burial 12, scale applies to all pictures: (a,b) Contaminant semi-spherical starch grain, from sample one, under normal and cross polarized light respectively; (c,d) Semi-spherical starch grain, from sample one, with a double faceted base under normal and cross polarized light respectively; (e,f) Damaged convex-lenticular starch grain, from sample one, under normal and cross polarized light respectively; (g,h) Contaminant small possible starch grain from sample two under normal and cross polarized light respectively.

### 35-1

Khuzhir-Nuge XIV Burial 35-1 was an older juvenile, aged 18-20, and a probable male. Two subsamples, originating from a sample removed from the distal interproximal surface of the right maxillary central incisor, were examined because the first subsample was deemed to be less

than representative of the whole sample. Sample one contained a small amount of fungal hyphae and spores. One possible starch grain was observed and found to be much like the possible grain from Khuzhir-Nuge XIV Burial 12, sample two (Figure E-46a, b). It was similarly eliminated as a contaminant.

Sample two contained more hyphae, though no spores were noted. Two starch grains were observed. One was concave-lenticular in shape, 18.9  $\mu\text{m}$  in length, and attached to a small amount of surrounding calculus material (Figure E-46c). Five to six rings of concentric lamellae were present, and the arms of the extinction cross were wide and dull. Birefringence was weak and inconsistent, showing signs of damage (Figure E-46d). This grain was considered distinct from control starch grains and therefore a non-contaminant. The other starch grain was smaller, roughly circular in shape, with a central hilum and a small indentation in surface at this feature (Figure E-46e, f). This starch grain looked quite similar to some observed in comparative samples of *Zea mays* and may have been a contaminant; it was removed from analysis as a precaution. One blue fiber was found in each subsample as well, further indicating that some contaminants entered the sample.

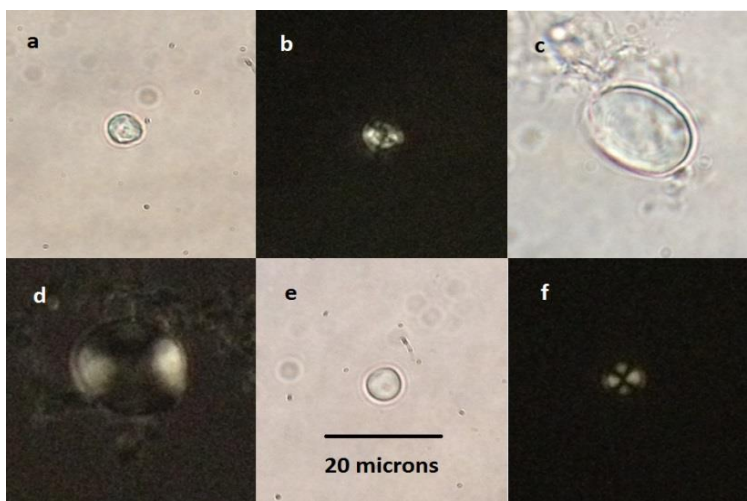


Figure E-46. Starch grains recovered from calculus associated with Khuzhir-Nuge XIV Burial 35-1, scale applies to all pictures: (a,b) Possible starch grain, from subsample one, with a likely extinction cross under normal and cross polarized light respectively; (c,d) Large concave-lenticular starch grain with visible lamellae, from subsample two, under normal and cross polarized light respectively; (e,f) Small contaminant round starch grain, from subsample two, under normal and cross polarized light respectively.



The subsample from Khuzhir-Nuge XIV Burial 34, an adult male, aged 25-35, originated from the lingual and distal surfaces of the left mandibular second premolar. It contained fungal spores and hyphae. It also integrated some probable pollen aggregates, similar to those seen in samples from Lokomotiv, attached to the demineralized calculus (see Section 5.2.4). One possible starch grain measuring less than 5  $\mu\text{m}$  was found, similar to the ones seen in Khuzhir-Nuge XIV Burial 12, sample two and Khuzhir-Nuge XIV Burial 35-1, subsample one. It could not be rotated, and only one to two possible arms of an extinction cross were visible. Similar small, featureless starch grains were found in control swabs and this grain was therefore considered a contaminant as a precaution.

Khuzhir-Nuge XIV Burial 11 was an adult male, aged 35-50. Two samples were originally taken from this individual: one from the lingual surface of the right mandibular second molar (sample one) and one from the mesial surface of the right mandibular central incisor (sample two). Sample two consisted of a small deposit attached to a loose enamel fragment, and it was placed in a sterile micro-centrifuge tube in its entirety in the hopes that the calculus sample could be scraped off in a more sterile environment at the University of Saskatchewan. Since some controls in the University of Saskatchewan lab also yielded small numbers of starch grains, it was decided not to examine this sample and instead to archive it for future work.

Sample one was analyzed and a subsample yielded a number of particles. Fungal hyphae and spores were noted throughout. No phytoliths were found, but three starch grains and a fourth possible starch grain were identified. One was quite small, measuring 6.1  $\mu\text{m}$ , and had a polyhedral shape, with a small indentation at the central hilum (Figure E-47a, b). An almost identical starch grain in shape, size, and birefringence was found within a mandible swab from Ust'-Ida I Burial 14-1-1 (Appendix D, Figure D-4t) and the grain was therefore considered a contaminant. Another was roughly oval in shape with an uneven surface and central hilum (Figure E-47c). It was 18.2  $\mu\text{m}$  in length. The birefringence of the starch grain was inconsistent, and some large fissures or cracks were present on the surface, indicating damage, including possible gelatinization indicated by slight swelling of part of the grain surface and birefringence disruption (see Chapter 5, Section 5.2.2; Figure E-47d). It was distinct from controls and

therefore kept within the analysis. The third starch grain was a discoid to slightly concave-lenticular shape, measuring 15.8  $\mu\text{m}$  (Figure E-47e). Again, the birefringence of the starch grain was uneven and showed there was damage to the starch grain (Figure E-47f). The arms of the extinction cross did not meet in the middle of the grain. Swelling was not noted for this grain. It likely had a central hilum, but this could not be confirmed due to the damage. Faint concentric lamellae were present. It was also considered distinct from controls. The fourth particle was quite unique in its characteristics, unlike anything seen in other samples or controls. It appeared to consist of two granular pieces (Figure E-47g). One piece was roughly rectangular in shape and opaque. This piece showed a cross under cross polarized light, consistent with starch grains (Figure E-47h). The other section was squarer in shape and slightly darker colored. It did not appear to have a similar cross under cross polarized light. Both pieces together measured 31.5  $\mu\text{m}$ . It may have been a complex starch grain or it may have been a particle or part of a particle with one component that mimicked an extinction cross.

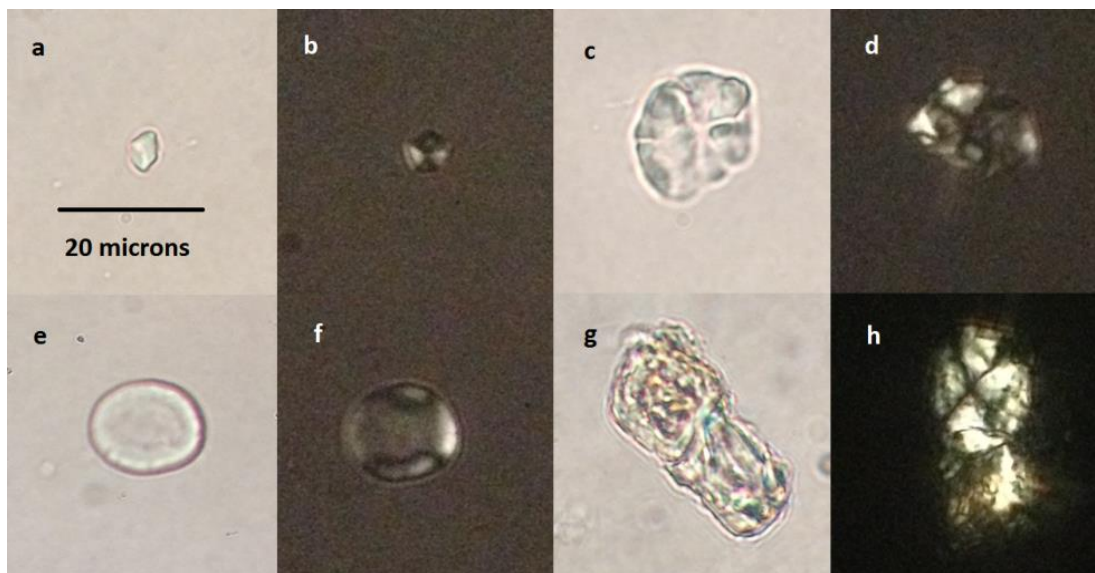


Figure E-47. Starch grains found in calculus associated with Khuzhir-Nuge XIV Burial 11, scale applies to all pictures: (a,b) Small contaminant angular starch grain under normal and cross polarized light respectively; (c,d) Damaged oval starch grain under normal and cross polarized light respectively; (e,f) Damaged flat to slightly concave-lenticular starch grain under normal and cross polarized light respectively; (g,h) Possible complex starch grain under normal and cross polarized light respectively.



Khuzhir-Nuge XIV Burial 32 was an adult female, of more than 50 years of age. The sample was taken from the buccal surface of the left maxillary second molar. Abundant hyphae and fungal spores were evident in the subsample. Three starch grains were noted, with faint extinction crosses. Only one could be rotated and was seen to have a polyhedral shape and central hilum (Figure E-48a, b). The extinction cross arms were sharp. This starch grain was similar to the contaminant polyhedral grain observed from the Khuzhir-Nuge XIV Burial 11 sample, albeit slightly smaller, and was eliminated from analysis as a precaution. The other two starch grains identified were almost identical in characteristics. They were likely roundish, including one that was attached to demineralized calculus (Figure E-48c-f). They both likely came from the same source and therefore both remained in the analysis. All three were less than 5  $\mu\text{m}$  in size. A black-brown mass may represent another piece of charcoal (see Section 5.2.5).

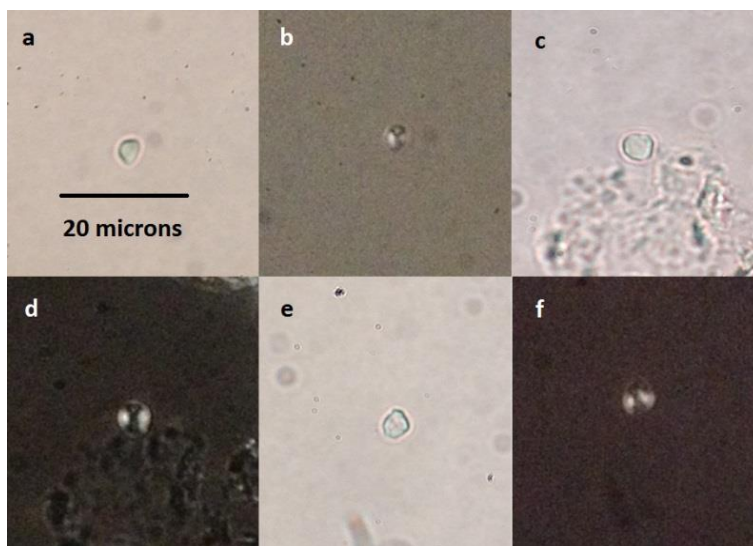


Figure E-48. Starch grains recovered from calculus associated with Khuzhir-Nuge XIV Burial 32, scale applies to all samples: (a,b) Contaminant polyhedral starch grain under normal and cross polarized light respectively; (c,d) Roundish starch grain attached to demineralized calculus under normal and cross polarized light respectively; (e,f) Almost identical starch grain under normal and cross polarized light respectively.

## **Appendix F: Microparticle Shape Descriptions**

Table F-1 Descriptive Terms for Starch Grains and Phytoliths Identified Within Dental Calculus

Characteristics	Description
“Chef’s hat”	Bulbous at one end with the other end more constricted and generally flat
Concave-lenticular	Lens shaped with one concave surface and one convex surface (like a thick contact lens)
Conical-flask	Cone shape with a flat base at one end while the other is a short cylinder shape (like an Erlenmeyer flask)
Convex-lenticular	Lens shaped with two convex surfaces meeting at acute points
Cylindrical	Rounded in the middle but with flat ends
Discoid	Similar to a lenticular shape but upper and lower surfaces are flat rather than concave or convex
Lobed	Divided into round or angular segments separated by a fissure
Oval	Oblong sphere
Polyhedral	Many plane faces present
Pyramidal	Having three sides coming to a point at one end and a flat roughly square base at the other end; with sharp edges
Rectangular	Elongated cube with flat surfaces
Rod	Long and narrow rectangle, edges can be smooth or sharp
Round	Spherical; no edges or protrusions to grain
Rounded-pyramidal	General pyramid shape but with rounded edges
Roundish	Almost spherical but slightly asymmetric
Semi-circular	Roughly half of a sphere with a flat base
Trapezoidal	Cuboid but with differing side lengths
Trichome-like	Shaped similar to a hair, with one thicker end

Characteristics	Description
	and another that comes to a point